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NOTICES

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ROOT DEVELOPMENT IN SOME COMMON NEW ZEALAND PASTURE PLANTS

VIII. THE RELATIONSHIP OF TOP GROWTH TO ROOT GROWTH IN PERENNIAL RYEGRASS (*Lolium perenne*), ITALIAN RYEGRASS (*L. multiflorum*) AND TALL FESCUE (*Festuca arundinacea*)

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(Received for publication, 16 February 1956)

Summary

The relationship between root growth and leaf yield in three grasses is discussed. Leaf yields per plant declined from their peak values in summer and followed the accepted growth patterns of low winter yield and spring recovery. For the early (establishment) period of growth, tiller numbers increased (with the exception of Italian ryegrass from February to April). The latter part of the period of the trial showed negative correlations between leaf yield and tiller numbers which can be best explained by the phenomenon of apical dominance.

There was a significant negative correlation in the ryegrasses between "white" roots and leaf yield per plant, but a lack of significance between "other than white" roots and leaf yield.

INTRODUCTION

In many of the studies that have reported upon the behaviour of above-ground parts of grasses, the root system has been completely ignored, while some root studies have not taken top growth into account. Such divorcing of the component parts of the plant gives a very incomplete picture of its functioning, for under normal growth conditions, all parts of the pasture plant are in physiological balance.

This paper reports further on the experiment of which the root behaviour has already been described (Jacques and Schwass, 1956), and outlines, firstly, the yield and the tillering behaviour of the top growth, and secondly, the relationships between top growth and the root system.

REVIEW OF LITERATURE

Top Growth

The best utilization of pasture growth is the basis of modern grassland techniques and it is complicated by the seasonal growth factor.

The seasonal nature of top growth in grasses and the control which can be imposed by various environmental factors have been well established by numerous workers.

In New Zealand, the seasonal production of pasture growth under various experimental conditions at Marton was studied by Hudson, Doak, and McPherson (1933). Department of Agriculture trials of grassland production and the rate of growth and yielding capacity of some of the major pasture species have been reported by Elliott and Lynch (1942), and more recently by Lynch (1949). With perennial ryegrass at Marton, Lynch found that growth usually commenced in early September, was at a maximum in October and November, fell away from December until February, and recovered during March or April to levels largely controlled by rainfall. Perennial ryegrass showed great variability between years in its summer and autumn production. Corkill (1950) showed that in the first 18 months after autumn sowing at Palmerston North, perennial ryegrass produced more top growth during summer and autumn than Italian, but less during winter and spring.

The effects on root and top growth of cutting the herbage in perennial ryegrass and timothy were reported by Roberts and Hunt (1936), and in perennial ryegrass and cocksfoot by Jacques and Edmond (1952). The latter studied the effect of various treatments on yields of top growth, and found that yields were greater from (a) fortnightly cutting than weekly cutting, (b) 2-in. height of cutting than 1-in., and both these heights than ½-in. height of cutting, and (c) non-root-pruning than root-pruning. The effect on yield from root pruning was greater than from height of cutting, which in turn had a greater effect than frequency of cutting. It was also found that, in general, the more lenient top treatments were associated with earlier spring growth and with better growth into the summer. From a study of the effects of clipping the top growth of cocksfoot, brome grass, ladino clover, and alfalfa, Wagner (1952) concluded that damage to top and root growth was less severe in seedlings than in older plants.

Relationship Between Herbage and Roots

Little concerning the relationship between herbage and roots has been reported in the literature, and most of this is incidental. Sprague (1933) worked with *Poa pratensis* and *Agrostis vulgaris* and found that, following the period of maximum root weights in early spring, a decrease in root weights occurred in both species at the time of heavy top growth. In *Poa pratensis,* there was a gradual recovery in root weight as top growth became less abundant. Roberts and Hunt (1936) found that the greatest root weight in perennial ryegrass and timothy was reached before the highest herbage weight. Stuckey (1941) reported that in ten grass species, which included unclipped perennial ryegrass, the peak of root growth took place in early spring before the top growth approached its maximum.

Working with some American range-grass species, McCarty and Price (1942) found that carbohydrate storage by grasses is cyclic in nature. Minimum storage coincided with maximum top growth, while maximum storage occurred in the autumn at the completion of secondary herbage growth. Troughton (1951) in Wales found that in permanent pastures where perennial ryegrass, *Poa trivialis,* and *Agrostis* spp. were

co-dominants the amount of root material was lowest in November with a consistent increase until May or June, and was then followed by a steady decrease. Jacques and Edmond (1952) showed that the maximum production of herbage and new roots in perennial ryegrass and cocksfoot did not coincide. Root initiation after March planting was at its lowest in early June as herbage production was falling, but root numbers increased from June as herbage continued to decline. Maximum root initiation was reached in October at a time when an increase in herbage growth was becoming apparent. Root numbers then fell away sharply until the lowest point was reached in December, and there was a slight recovery in January as herbage growth declined.

Materials and Methods

Seedling plants of the three grasses under trial were planted out in pipes on 13, 14, and 15 August, 1952. Establishment of all plants was good, a first cut of top growth was taken on 17 September, and thereafter at approximately fortnightly intervals until the cut on 16 April 1953. By this time, leaf yields had decreased markedly as temperatures fell during late autumn, and the cutting interval was extended to 3 weeks to avoid undue interference with root behaviour. The final top cut was taken on 19 October 1953.

The trial comprised 17 blocks each of 8 pipes which were lifted at intervals for root studies. There were 4 plants per pipe which allowed 8 plants of each species in each block (*Poa annua* was included in the layout, but was discarded early in the trial because of heavy mortality). The herbage when cut was divided into two samples, 8 blocks being included in the first and 9 blocks in the second, depending on the position of blocks in the experimental layout. As the first lifting took place on 4 December 1952, the second on 29 December, and the rest in succession at 3-weekly intervals during 1953, the number of plants cut decreased accordingly from 136 for each species in the early cuts to 16 at the final cut. The two cutting samples provided a check against gross errors, but did not allow a detailed statistical analysis of leaf yield to be made.

All above-ground tissue was cut 2 in. from the crown of the plant. The green weight of this was recorded and duplicate determinations of the dry-matter percentage were made for each sample. Drying was carried out at a temperature of 100°C. for 24 hours, a method recommended by a committee of the New Zealand Institute of Chemistry for plant tissues such as leaves which are low in soluble carbohydrate (Melville 1947). The yields of dry matter were calculated from these figures, and have been recorded in the results as the yields of dry matter per plant per day, reduction to this basis compensating for the decrease in plant numbers as they were lifted, and to some extent for the lack of strict 14- or 21-day cutting intervals.

Emerging flowerheads were recorded in some of the plants of each species despite the fairly intense cutting treatment over the summer. The cutting dates at which more than 25% of the plants present possessed developing flowerheads were as follows:—

Perennial ryegrass:	28 November 1952 to 21 January 1953.
Italian ryegrass:	6 January 1953 to 21 January 1953.
Tall fescue:	11 December 1952 to 4 February 1953.

In addition, more than 10% of the tall fescue plants were recorded as flowering from the cut on 18 February until the cut on 20 May 1953.

The cutting height of 2 in. avoided the inclusion of basal stem tissue in the yield of top growth, but, during the above periods, appreciable amounts of flowering stem tissue were included with the leaf tissue in the yield of cut herbage.

Tiller numbers were counted for each plant when its appropriate block of pipes was lifted for the root determinations. Some green tissue had to be present for the tiller to be counted as alive, but it was not found feasible to place tillers into size groups.

RESULTS

Leaf Yields

The leaf yields and the dry-matter percentages for each species are set out in Table 1.

Italian ryegrass made rapid early growth and reached a high level of production in early November, which lasted until the end of December; thereafter production declined.

Perennial ryegrass was slower in its early growth, came to a high level of production at the same time as Italian but maintained it to early March, after which production fell away, and from May onwards was below that of Italian ryegrass.

Tall fescue was the slowest to start active growth, but steadily increased until it produced a peak leaf yield during January of over 200 mg. of dry matter per plant per day, which is more than twice as much as that produced by either of the ryegrasses. Tall fescue steadily declined to a minimum during July and August, but recovered in September and the yield was still increasing at the completion of the trials. It outyielded perennial ryegrass from mid November and Italian ryegrass from the end of November to the end of the trial. The high yields given by tall fescue under the fairly intensive cutting treatment of this experiment, and the short low-production period in winter, are noteworthy features.

There was a general inverse relationship between leaf yield and leaf-dry-matter percentage, in that the percentage was lowest when the plant was actively growing and rapidly forming new leaf tissue and highest when growth was slow. Correlation coefficients were calculated for dry-matter percentages and dry-matter yields and showed a negative correlation which reached significance at only the 5% level in perennial ryegrass and tall fescue, but did not reach significance in Italian ryegrass.

The dry-matter percentages of Italian ryegrass herbage were consistently below those of perennial ryegrass by some two to four per cent. The values determined for tall fescue fluctuated over the trial period, resembling those for Italian ryegrass until April and from early September, but approximating those for perennial ryegrass over the winter period.

Tillering

The mean numbers of live tillers per plant in each species as determined at each block lifting date are set out in Table 2. An analysis

TABLE 1. LEAF YIELDS PER PLANT PER DAY AND DRY-MATTER PERCENTAGES

Date	Perennial Ryegrass		Italian Ryegrass		Tall Fescue	
	Leaf Yield mg.	Dry Matter %	Leaf Yield mg.	Dry Matter %	Leaf Yield mg.	Dry Matter %
1952						
1 Oct.	25	19.9	46	17.4	14	18.3
15 „	49	18.6	70	16.8	18	16.8
30 „	77	19.8	73	18.1	51	17.2
13 Nov.	90	21.3	92	18.9	77	18.3
28 „	71	21.0	64	17.6	91	18.6
11 Dec.	90	20.2	86	17.3	142	18.0
22 „	97	20.4	94	17.6	161	18.5
1953						
6 Jan.	95	21.7	67	18.4	179	19.4
21 „	92	22.6	66	20.4	208	20.8
4 Feb.	83	21.2	47	17.5	169	18.2
18 „	83	21.2	60	17.4	178	18.1
4 March	80	20.8	47	17.3	150	17.6
18 „	51	23.6	39	19.2	112	19.6
2 April	47	23.7	40	19.8	117	19.6
16 „	38	23.4	31	19.5	74	20.4
4 May	42	23.2	41	19.6	75	21.6
20 „	31	23.3	35	19.3	48	20.5
8 June	23	22.8	29	19.0	39	22.3
2 July	21	25.6	30	20.9	28	24.8
23 „	17	23.2	24	19.4	28	24.1
14 August	17	25.0	23	20.0	34	23.7
8 Sept.	16	23.9	27	19.4	74	20.6
23 „	27	20.7	33	17.2	128	18.6
19 Oct.	24	19.7	22	16.9	148	17.4

of variance on these data was calculated and the results showed a highly significant interaction between dates and species, indicating that differences between the species did not remain constant over all lifting dates. A separate analysis of variance was recalculated for each lifting date and the differences between the mean numbers of tillers required for significance at both the 5% and 1% levels are given in columns 4 and 5 in Table 2.

In Italian ryegrass, tiller numbers rose to a minor peak of about 60 at the end of January, and then declined to a little over 30 at the end of March. This points to a considerable mortality among the tillers

TABLE 2. MEAN NUMBERS OF TILLERS PER PLANT FOR EACH SPECIES AT EACH LIFTING DATE

Date	Perennial Ryegrass (1)	Italian Ryegrass (2)	Tall Fescue (3)	Differences Required for Significance	
				5% level (4)	1% level (5)
1952					
4 December	78.0	25.0	50.3	14.4	19.5
29 „	66.6	40.3	46.4	13.9	19.0
1953					
26 January	84.0	59.6	43.1	20.6	28.0
16 February	105.8	37.8	54.5	35.0	48.1
9 March	143.9	40.4	52.5	37.6	51.2
30 „	147.8	32.4	64.0	37.7	51.5
20 April	101.5	35.8	59.0	30.4	41.3
11 May	146.3	55.4	67.3	48.9	66.5
1 June	125.4	62.6	72.3	40.0	54.6
22 „	108.6	68.3	89.1	38.1	51.8
13 July	138.5	116.6	101.0	47.5	65.0
3 August	145.8	103.2	79.1	43.9	60.2
24 „	136.1	103.6	93.3	53.2	72.5
14 September	73.3	65.5	75.4	38.5	52.6
5 October	128.0	57.7	90.9	40.1	54.6
27 „	74.3	70.6	83.3	40.8	55.5
16 November	84.4	28.1	81.4	18.8	25.6

during the autumn period when leaf growth is slowing down. Tiller numbers increased from the end of April, and reached a peak value of near 120 in the middle of July, but the number declined after that date.

In perennial ryegrass, the mean numbers of live tillers per plant had reached more than 140 by early March, and thereafter the trend was for high tiller numbers until October. It seems possible that during this seven-month period the tiller numbers reached saturation point for the pipes; in other words, a total of some 560 to 580 tillers was the maximum number that the soil in one pipe was capable of supporting with water and nutrients.

The mean leaf yield of an individual tiller of each species was calculated, and the yield is shown graphically in Fig. 1. The dates of top cuttings and liftings for root and tiller counts did not coincide. The data used for these calculations were the tiller numbers as counted for each lifting date, and the leaf yield for these same dates interpolated from a leaf-yield graph. The figure shows that the peak yield in Italian ryegrass was reached at or before the first lifting on 4 December, while the peaks for perennial and tall fescue were reached at a later date. In perennial ryegrass, the leaf yield of an individual tiller was only some

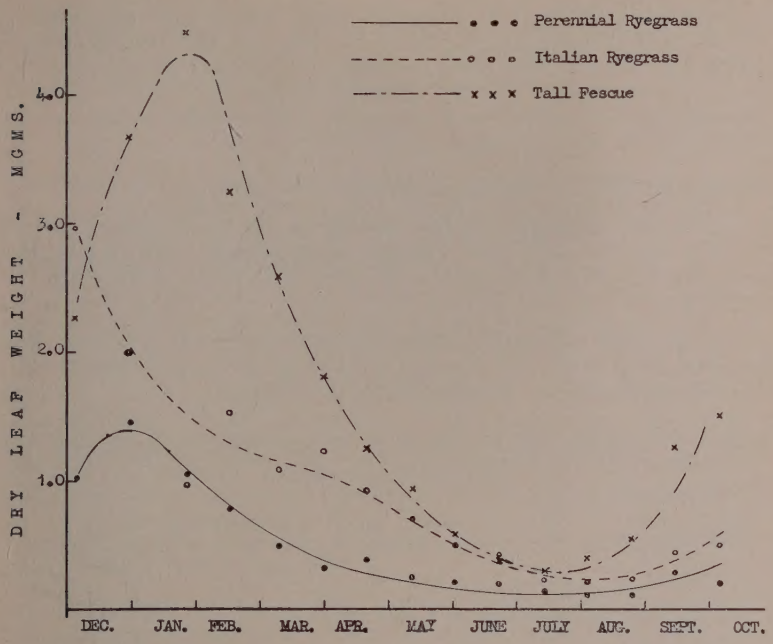

Fig. 1.—Dry leaf yield per tiller in three grass species.

50% to 60% of that of Italian. The yield curve for tall fescue illustrates the two features already mentioned; the spectacular high peak for yield and the short winter-growth trough.

Correlation coefficients were calculated for the mean number of tillers per plant and leaf yields per plant; the results are set out in Table 3.

TABLE 3. CORRELATION COEFFICIENTS FOR MEAN NUMBER OF TILLERS PER PLANT AND DRY WEIGHT OF LEAF PER PLANT PER DAY
4 December 1952 to 5 October 1953

Species	Calculated Value	Values Required		Result
		5% level	1% level	
Perennial ryegrass	— 0.577	0.514	0.641	*
Italian ryegrass	— 0.647	,,	,,	**
Tall fescue	— 0.728	,,	,,	**

In Italian ryegrass and tall fescue, the negative correlation coefficients attained significance at the 1% level, but significance was only reached at about the 3% level in perennial ryegrass. This reduced level of significance in perennial can be explained by the rapid increase of tiller numbers to a maximum in early March which was maintained for several months, while the leaf yield curve declined from late January

to early June, and was at minimum values during July and August. The result of this test in each species shows that, as leaf yields decreased during the autumn and winter periods, the numbers of live tillers in each plant increased (with the observed exception of Italian tillers in autumn), and that tiller numbers declined again in spring as leaf growth commenced for the new season.

Relationships between Herbage and Roots

The root behaviour in this experiment has been reported in the previous paper of this series (Jacques and Schwass, 1956) where the mean numbers of "white" roots and "other than white" roots per plant are recorded.

Correlation coefficients were calculated between the mean leaf yield per plant, interpolated for the root block lifting dates, and the two root classes for each species. Results are set out in Table 4.

TABLE 4. CORRELATION COEFFICIENTS FOR MEAN NUMBERS OF "WHITE" ROOTS AND OF "OTHER THAN WHITE" ROOTS PER PLANT, AND DRY WEIGHT OF LEAF PER PLANT PER DAY

Root Class and Species	Calculated r Value	r Values Required		Result
		5%	1%	
"White" roots: (period 9 Mar. to 5 Oct. 1953)				
Perennial ryegrass	— 0.659	0.602	0.735	*
Italian ryegrass	— 0.805	„	„	**
Tall fescue	— 0.246	„	„	N.S.
"Other than white" roots: (period 4 Dec. 1952 to 5 Oct. 1953)				
Perennial ryegrass	0.142	0.514	0.641	N.S.
Italian ryegrass	0.035	„	„	N.S.
Tall fescue	— 0.470	„	„	N.S.

In both ryegrass species, a significant negative correlation existed between mean numbers of "white" roots per plant and leaf yields. During the period from early March until early October, "white" root numbers first increased as herbage growth declined into the winter, and, later, the "white" root numbers fell away as herbage yield recovered towards the end of the experiment. The time of maximum leaf growth did not coincide with the time of maximum root initiation in either ryegrass species.

In tall fescue, failure of the correlation coefficient to attain significance was most likely due to the fact that "white" root numbers were at a fairly uniform level during the entire period covered by the calculation, while leaf growth followed a pronounced trough. Root initiation in tall fescue was occurring steadily over the whole of the late autumn, winter, and early spring periods.

In all species, the lack of significance between leaf growth and numbers of "other than white" roots is worthy of note.

Correlation coefficients were also calculated between leaf yield per plant and mean dry weight of each root class per plant in each species. The results were very similar to those based on root numbers given above, and the same conclusions can be drawn.

Discussion

Despite largely unexplained "peaks and troughs" in the values for each character considered in this paper (which are evident in the tables) the trends in each component of the negative correlations that were established behaved in the expected manner.

Leaf yields per plant in each species declined from their peak values in summer and followed the accepted growth patterns of low winter-yield and spring recovery. The numbers of tillers per plant in each species, with the exception of Italian ryegrass, showed the increases that would be expected during the first few months of establishment from August plantings. In Italian ryegrass, tillers died at a rate faster than their replacment from February to April, and under competitive pasture conditions this mortality may be a cause of failure of many plants to survive into winter. Careful management of both Italian ryegrass (and also of short-rotation ryegrass of which Italian is one parent) is necessary under field conditions in autumn if these species are to produce leaf freely over the following winter.

The period covered by the negative correlations between leaf yields and tiller numbers (Table 3) can best be explained by, and fits into, the phenomenon of apical dominance. High values for both these characters following their normal establishment growth were reached in all species before the end of March. As leaf growth declined during the autumn and through the winter, the demand for metabolites by the main central tillers was much reduced, and some became available to initiate and support small lateral tillers. In the present experiment, all live tillers bore some green photo-synthetic tissue but many of them were very small and contributed little to yield. With the recovery of leaf growth in the spring as temperatures increased, the existing main tillers made a strong demand for metabolites, with a resultant tendency towards starvation and suppression of the weaker tillers. The tiller mortality which became evident as plants came into the spring was probably due to the above factor together with the death of some older tillers.

The small size of the winter tillers may also be due in part to direct environmental influence upon leaf size. Peterson and Loomis (1949) showed that, in *Poa pratensis*, lowering the temperature and shortening the daylength reduced the length of leaves. With cocksfoot, Stuckey (1942) induced significantly longer leaves in vegetative plants grown in 16-hour days than in those grown in 8-hour days. Langer (1954) has reported that, in timothy, warmer temperatures and longer day-lengths speeded up the rate of leaf appearance and the rate of leaf elongation and increased the final leaf length. Similar effects have been recorded by Mitchell (1953a) in ryegrass.

In a study of young vegetative ryegrass plants reported by Mitchell (1953b), such treatments as shading, reduction of the period of daily illumination, exposure to high temperatures, and to a lesser extent partial defoliation, reduced lateral tiller formation. Since such treatments also

control either the potential rate of formation or rate of utilization of energy substrate for growth or respiration, Mitchell concluded that, in ryegrass, the development of lateral tillers can be regulated by the general level of energy substrate in the plant.

The winter period has lower temperatures, shorter daylengths, and lower light intensities than summer. Under the conditions of the present experiment, the lower temperatures could probably offset the effects of reduction in light intensity upon the energy substrate levels in the plant (c.f. Mitchell, 1955). During the winter, lateral tiller formation can proceed apace, although the size of the individual tillers will be restricted by such factors as the general low levels of metabolic activity at that season, and the effects of the winter environment on leaf length.

At the end of December, the leaf yields per tiller showed considerable differences between the species, and tiller numbers per plant also showed differences at this time. Tall fescue had the highest leaf yield per tiller of approximately 4 mg. of dry tissue per day, and the mean tiller number per plant was about 50. In Italian ryegrass, the leaf yield per tiller was about 2.5 mg. per day with some 60 tillers, while perennial ryegrass produced nearly 1.5 mg. of dry leaf tissue per day with about 80 tillers per plant. A similar relationship between the ryegrasses existed in winter also, when Italian was producing nearly double the amount of leaf per tiller from rather less than half the number of tillers per plant than perennial.

The significant negative correlations in the ryegrass between "white" roots and leaf yield per plant demonstrates the differential seasonal growth between leaf tissues and root system. It confirms an observation made in perennial ryegrass and cocksfoot and stated by Jacques and Edmond (1952) as follows: "The periods of maximum production of roots and tops did not coincide . . . As root and shoot behaviour was comparable for all treatments, it would appear that the alternation between root and top growth was not in response to any stimulus developed in this experiment, but rather, was a response to stimuli affecting normal physiological development."

During the winter, herbage yields are at low levels while new root initiation and the formation of an active root system is proceeding vigorously. The emphasis in the plant at this stage is towards building-up a root system and food reserves in storage tissues which will be capable of supplying the great demands for nutrients made by the spring leaf growth and by subsequent flowering. This winter activity can be modified by environmental conditions; a common observation is that spring leaf production following autumn-saved pasture fed off in late winter is both earlier and more vigorous than that from a pasture which has been hard grazed in winter. The high levels of spring herbage growth are a function of the newly formed "white" roots, which are very efficient in the uptake of metabolites and water. Older roots persisting from the previous growth season have lost cortical tissue over much of their length and are less efficient (Jacques and Schwass, 1956). This is supported by the lack of significance shown in the present experiment between numbers of "other than white" roots and leaf yield (Table 4).

The relationship between top growth and root growth underlies some of the recommendations made for grazing management on American range country. McCarty and Price (1942) showed that early spring

growth is dependent on the presence of adequate carbohydrate reserves, and the amount of foliage present during the normal storage period determines the amount of these reserves accumulated in roots and stem bases. These workers recommend that grazing should be slackened during the critical periods in the grasses' life cycle: (a) during the active reproduction stage from heading to seed ripeness when plant reserves are at a minimum, and (b) during the early part of the normal storage period when undue interference may leave insufficient time for complete regrowth and build-up of reserves.

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EFFECT OF FOLIAR AND SOIL APPLICATIONS OF INSECTICIDES ON CONTROL OF APHIDS AND TURNIP-MOSAIC VIRUS ON SWEDES

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Summary

In a glasshouse trial lindane and parathion were effective against *Myzus persicae* on swedes two days after application but not after seven days.

In the field, spray applications of lindane, parathion, schradan, TEPP and soil treatments of schradan significantly reduced naturally occurring aphid populations. All materials reduced the incidence of turnip-mosaic virus, parathion being the most effective. Correlation was apparent between aphid numbers and virus incidence and between incidence of virus and yield.

Introduction

The effectiveness of some organic phosphorous insecticides in reducing populations of the aphid *Myzus persicae* Sulz. on swedes (*Brassica napobrassica* D.C.) grown in the glasshouse was investigated by Lamb and Jacks (1954). The experiments described here were set up to compare the effectiveness of lindane (which was not used in the former trial) and parathion under glasshouse conditions and to ascertain the effect of lindane, parathion, schradan, and TEPP on naturally occuring aphid populations on swedes in the field.

Materials

The following insecticides were used:

Nexa (16% lindane)
Folidol (24% parathion)
Sytam (70% schradan)
Murphy Tepp (40% TEPP)

Experimental Methods and Results

Glasshouse Trial

A trial was carried out in the glasshouse to ascertain the effectiveness of lindane against populations of *M. persicae*. Parathion was used as a basis of comparison and a water spray as control. Swede plants, variety Superlative, growing in potting soil in six-inch pots were sprayed with 0.025% lindane, 0.024% parathion, or water. At intervals of two, seven, nine, ten, fourteen, and sixteen days after spraying, twenty apterous *M. persicae* were added to each of three plants treated with each spray material. Twenty-four hours later the survivors were counted. From Table 1 it may be seen that aphids added two days after spraying failed to survive on plants sprayed with lindane or parathion, but after seven days neither insecticide was effective.

Field Trial

A field trial to compare the effectiveness of materials listed in Table 2 was made on an area with a history of a high incidence of

turnip-mosaic virus. A randomized block design with six replications for each of 13 treatments was used. Each replicate was a four-row plot with 18 in. between rows. Plants were thinned to six inches apart in the rows giving ten plants per row. Spray materials were applied at intervals of 7, 14, or 21 days as shown in Table 2. Soil applications

TABLE 1. DURATION OF EFFECTIVENESS OF INSECTICIDES AGAINST *Myzus persicae* ON SWEDES

Number of aphids per plant 24 hours after addition of 20 adults.

Treatment	Days between spray application and addition of aphids					
	2	7	9	11	14	16
Water	9.6	19.3	13	28	26	17
Lindane 0.025%	0	13.3	14.6	14.6	20.6	17
Parathion 0.024%	0	15.6	11	18	20.6	20

TABLE 2. EFFECT OF INSECTICIDES ON APHID POPULATION, VIRUS INCIDENCE, AND YIELD OF SWEDES

Treatment	Number of Aphids		Virus Incidence		Yield per Plant (lb.)
	Mean 16 Plants	Log (n + 1)	%	Angle	
Spray applications					
Water 7 days	955	2.90	94.5	79.9	0.69
Lindane 0.025% 7 days	12	1.00	57.2	49.4	0.93
Lindane 0.025% 14 days	73	1.80	55.2	48.2	0.79
Parathion 0.024% 7 days	11	1.01	33.0	34.8	1.04
Parathion 0.024% 14 days	111	1.98	51.5	45.8	0.95
TEPP 0.025% 7 days	54	1.49	55.6	48.8	0.86
Schradan 0.35% 7 days	11	0.95	60.4	51.2	0.74
Schradan 0.35% 21 days	71	1.78	43.3	41.1	0.94
Schradan + TEPP 7 days	11	0.97	53.3	47.1	0.97
Schradan + TEPP 21 days	115	2.04	72.9	60.2	0.73
Soil Watering					
Schradan 0.35% 7 days	4	0.64	47.9	43.9	0.85
Schradan 0.35% 14 days	13	1.00	60.7	51.5	0.99
Schradan 0.35% 21 days	25	1.35	47.0	43.3	0.96
Sig. diff. 5%		0.39		13.9	0.25
1%		0.52		18.5	0.34

of schradan were made at the rate of 20 fl. oz. per row before plants were thinned, and then at four fl. oz. per plant, at intervals of 7, 14, or 21 days. Treatments were commenced on 21 March 1955, when plants were in the cotyledon stage.

By 2 May, when two series of treatment applications had been made, aphids were sufficiently numerous for differences between treatments to become apparent. Aphids were counted on four plants in each row, a total of 16 plants per replicate. Almost all were apterous *M. persicae* with only an occasional alate form. Alate and apterous *Brevicoryne brassicae* were rarely found. Application of treatments continued, and records of virus incidence were taken a month later, when plants were pulled and weighed. Results are given in Table 2.

Effect of Aphid Population. Aphid populations on all treatments were significantly smaller than on control plots. Schradan watered on the soil at intervals of 7 to 14 days controlled aphids to the same extent as weekly sprays of lindane, parathion, schradan, or schradan plus TEPP which were equally effective and significantly better than 14- or 21-day applications, or weekly applications of TEPP.

Virus Incidence. Incidence of virus was lower than control with all treatments, but the difference between treatments did not correspond in all cases to difference in aphid numbers. Over all treatments, however, there was a significant correlation between aphid numbers and virus incidence of $r = 0.69$ ($P = 0.01$) showing that the amount of virus infection was related to the numbers of aphids present.

Effect on Yield. From the mean weight per plant given in Table 2 it is apparent that only 7-day spray applications of parathion gave a yield increase significant at the 1% level. Sprays of parathion applied every 14 days, TEPP every seven days, schradan every 21 days, and schradan watered on the soil at 14- or 21-day intervals were significantly better than control at the 5% level. Lindane at 14-day intervals, schradan at 7-day intervals, and schradan plus TEPP at 21-day intervals gave significantly lower yield than parathion at 7-day intervals ($P = 0.05$).

The correlation over all treatments between aphid numbers and plant weight was $r = -0.52$ ($P = 0.07$) and the partial correlation eliminating effect of virus was $r = -0.02$. There was therefore no direct relation between aphid numbers and yield. Correlation between virus and plant weight, $r = -0.75$ ($P = 0.003$), however was significant as also was the partial correlation, $r = -0.63$ ($P = 0.03$), when effect of aphid numbers was eliminated.

Conclusion

In glasshouse tests the effectiveness of lindane was of the same order as that of parathion for which the result obtained was similar to that of Lamb and Jacks (1954), who found that the residual effect of this compound did not extend beyond two days.

Results of the field trial show that all insecticide applications effectively reduced the aphid population. Although in this trial aphids were not numerous enough to cause obvious plant damage, the result suggests that application of one of these insecticides should protect brassica field crops against damage by high aphid populations.

No information is available on the number of winged aphids present at any one time. The number of individuals recorded however, gives an indication of the number of winged arrivals that survived. Thus the correlation between aphid numbers and virus incidence should relate equally to aphids actually counted, or to the winged forms arriving from an external source or breeding within the trial area.

Virus incidence was significanty lower than control with all materials used, applications of parathion every seven days being the most effective. Although between 30% and 60% of plants were infected in the treated plots, the results show that incidence of the non-persistent turnip-mosaic virus can be reduced by insecticide applications.

Owing to the high toxicity of residual and systemic insecticides, care should be exercised when deciding to use them for application to crops intended for stock or human consumption.

ACKNOWLEDGEMENT

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SPRAY EXPERIMENTS TO CONTROL *Botrytis cinerea* ON TOMATOES

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(Received for publication, 15 November 1955)

Summary

Of fungicides tested against *Botrytis cinerea* on glasshouse tomatoes, thiram gave the most satisfactory control. Applied fortnightly, 0.1% and 0.2% concentrations were equally effective in reducing infection and increasing fruit yield.

Introduction

It has been shown (Newhook and Davison, 1953, 1956) that *Botrytis cinerea* Pers. attacking glasshouse tomatoes treated with fruit-setting hormones can be checked by addition of a fungicide to the hormone, followed by an application of the fungicide alone 10–14 days later. While infection of dead flower petals is a major source of inoculum for general infection of plants, the disease may also establish itself in a crop from other sources, such as plant debris, and there is often need for a spraying programme that will supplement the truss spray programme designed by Newhook and Davison. The present paper describes experiments to find a suitable fungicide for general application to indoor tomato crops for protection against grey mould.

Preliminary Trials

Small-scale tests of fungicides were made on potted tomato plants of approximately five weeks' growth. A burn was made, with a coiled red-hot wire, on each of 25 leaflets on each plant. Two days later, when the burnt tissue had dried, four plants were sprayed to saturation with each fungicide. After another 24 hours, when the spray had dried, a spore suspension of about 100,000 spores/c.c. prepared from pure cultures of *B. cinerea* was sprayed on the plants until they were completely wetted. Plants were then held for five or six days at 65–70°F. in 95–100% humidity and the resulting grey-mould lesions counted.

Fungicides were tested in groups, unsprayed controls being included with each. Although the conditions of testing each group were made as similar as possible, comparisons of numbers of lesions between groups is not strictly justified. Therefore, in Table 1 the number of infections after each treatment is given as a percentage of the number on the unsprayed plants in the same group. The percentage of infected burns on controls ranged from 88–100% in the different groups.

Table 1 shows the results of the preliminary experiments. Only thiram, captan, and ziram gave satisfactory control without adversely affecting the tomato seedlings. These were further tested in an experiment on a winter crop of tomatoes grown under glass.

Glasshouse Trial

Plants of the variety Bay State were grown in single rows of ten, with 3 ft. between rows and 15 in. between plants in a commercial-type glasshouse. Plants were set out on 4 March and were grown to five

TABLE 1.—GREY-MOULD INFECTION AFTER INOCULATION OF TOMATO LEAVES SPRAYED WITH DIFFERENT FUNGICIDES

Fungicides Effective		Fungicides Effective but Causing Damage		Fungicides Ineffective	
Material and Concentration	Infected Burns, % of control	Material and Concentration	Infected Burns, % of control	Material and Concentration	Infected Burns, % of control
Thiram 0.2%	2	* Dichlone 0.1%	2	* Maneb 0.28%	14
Thiram 0.1%	2	Dichlone 0.05%	0	Cupram 0.28%	82
Thiram 0.05%	3	† Ferbam 0.28%	0	Copper oxychloride 0.25%	97
Ziram 0.28%	0			Cuprous oxide 0.25%	97
Ziram 0.14%	0			Colloidal copper (8.5% Cu) 0.25%	100
Ziram 0.07%	0			Colloidal sulphur 0.16%	99
Captan 0.2%	0			Tetrachloroparabenzoquinone 0.2%	82
Captan 0.1%	2			Gliodin 0.135%	98
Captan 0.05%	1			Salicylanilide 0.075%	90
				‡ 36L 0.4%	97
				Zineb 0.13%	100
				Nabam 0.06% + $ZnSO_4$ 0.2%	49
				Pentachloronitrobenzene dust	100
				Tetrachloronitrobenzene dust	100
				Bordeaux mixture : copper sulphate 6 lb./hydrated lime 8lb./water 100 gal.	74

* Caused severe leaf scorch.
† Did not cause damage but left an unacceptable black residue.
‡ Proprietary name.

trusses. The experiment was completed on 18 August when the last of the crop was picked.

The experiment was laid out in four blocks of six single-row replicates, and the following fungicides were applied by knapsack sprayer: thiram 0.2% and 0.1%; ziram 0.28%; captan 0.2% and 0.1%. To each of these were added copper oxychloride 0.25%, to check late blight, and DDT 0.05% p.p.i. and nicotine sulphate 0.17%, to suppress insects. In each block, one control row was sprayed with copper oxychloride and insecticides. Sprays were applied on 8 and 22 April, 5 and 21 May, and 3 and 17 June, that is, at approximately fortnightly intervals until the plants had ceased growth.

The fruit-setting hormone β-naphthoxyacetic acid was applied at 60 p.p.m. to the flowers of each truss, combined with thiram, ziram, or captan, on plants being sprayed with these materials, at the concentrations used in the general sprays. Control plants received the hormone without fungicide. Application dates were as follows: first truss, 14 and 20 April; second truss, 20 and 27 April; third truss, 3 and 11 May; fourth truss, 25 May; fifth truss, 25 May and 2 June.

All plants were inoculated four times, on 28 April, 10 and 27 May, and 17 June, by spraying them lightly from a knapsack sprayer with spore suspensions from pure cultures of *B. cinerea*. This was done early in the evening on days of rain or high humidity, and the glasshouse was closed down until approximately midday of the following day.

By mid May, grey mould had become well established on control plants, and some lesions were present on the others. As the fruit matured, infected tomatoes were counted from each row, and the sound ripe fruits from each truss were counted and weighed. No late blight appeared, and there was only a small amount of leaf mould (*Cladosporium fulvum*), the variety Bay State being comparatively resistant to this later disease.

To measure the amount of grey-mould infection on the vegetative parts of the plants, the diseased tissue on the ten plants of each row was cut out, oven-dried, and weighed. The green weights of the uninfected parts of the plants, excluding roots, were taken. A sample of healthy tissue collected at random through the crop and weighing approximately 2,500 g. was weighed green, then oven-dried and the dry weight taken. From the dry/green ratio thus found, the green weight of healthy plant in each row was converted to dry weight, and the percentages, by dry weight, of grey-mould-infected tissue in each row calculated.

Results are set out in Table 2. An analysis was made of the truss × treatment interaction, but this was not significant.

The almost complete absence of other fungous diseases allows differences in weights and number of sound fruits to be regarded as resulting from differences in control of grey-mould infection on vegetative tissue, flowers, and fruits, combined with any direct effects the fungicides may have had on the plants. An improved yield must combine increase in weight with increase in number of fruit, since increase in one without corresponding increase in the other suggests departure from optimum fruit size. Losses due to both flower infection

TABLE 2. INCIDENCE OF GREY MOULD IN GLASSHOUSE TOMATOES RECEIVING DIFFERENT FUNGICIDES

Treatment	Diseased Vegetative Tissue		Infected Fruit		Sound Fruit	
	% (weight) Diseased	Mean Equiv. Angle	% (number) Infected	Mean Equiv. Angle	Mean Weight per Plant (lb.)	Mean Number per Plant
Control	15.7	23.30	23.0	28.63	2.83	15.1
Thiram 0.2%	* 5.9	14.02	* 3.7	11.15	* 3.74	* 19.9
Thiram 0.1%	* 4.1	11.66	* 4.9	12.76	* 3.97	* 20.6
Ziram 0.28%	7.6	15.95	*11.2	19.57	3.35	18.7
Captan 0.2%	* 6.0	14.14	15.6	23.24	* 3.65	17.0
Captan 0.1%	6.4	14.62	*12.1	20.38	3.37	16.5
Sig. diff. 5%		6.28		5.80	0.55	2.8
1%		8.69		8.02	0.77	3.8

* Significantly better than Control at 1% level.

and infection of developing fruit affect the numbers of sound fruits harvested. Tomatoes remaining on depleted trusses tend to be larger than those on full trusses, although this is offset in some degree by the depression of vigour in plants with extensive areas of diseased vegetative tissue. To be successful, a treatment must reduce infection of vegetative tissue to a minimum, and must allow the development to maturity of the maximum number of fruits for which the plants can provide. In the present experiment, by stopping the plants at the fifth truss, while they were still capable of vigorous growth, effects due to nutritional deficiencies were avoided. Of the three materials, only thiram caused a reduction of infection significant at P 0.01 in both fruit and vegetative tissue, together with a significant increase in fruit weight and number. The higher concentration had no advantage over the lower. Ziram, while lowering the amount of fruit infection, produced a poorer crop weight and allowed more infection on leaves and stems. Captan at 0.2% lowered infection on vegetative tissue and gave a significantly heavier crop than controls, but did not check fruit infection sufficiently. At 0.1%, captan depressed infection amongst fruits and caused a fair reduction of disease in vegetative tissue, but failed to make a significant improvement in yield.

CONCLUSION

From the experiments it appears that thiram is the most useful fungicide for controlling grey mould on tomatoes. Fortnightly applications of a 0.1% concentration gave satisfactory reduction of disease, and also increased fruit yield.

ACKNOWLEDGEMENT

Statistical analysis of results was done by Mr. W. B. Taylor of Applied Mathematics Laboratory.

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APPENDIX

Almost all fungicides used were in commercial preparations, as follows:

Thirospray (thiram); Zirospray (ziram); Esso Fungicide 406 (captan); Phygon XL (dichlone); Fermspray (ferbam); Manzate (maneb); Cuprox (copper oxychloride); Perenox (cuprous oxide); Fungex (colloidal copper); Colsul 40 (colloidal sulphur); Crag 341C (gliodin); Tetroc (tetrachloroparabenzoquinone); Dithane Z-78 (zineb); Dithane D-14 (nabam); Botrilex (pentachloronitrobenzene); Folosan (tetrachloronitrobenzene); Shirlan A.G. (salicylanilide).

A NOTE ON FIELD CONTROL OF CELERY LEAF-SPOT (*Septoria apii* Chester)

By H. Jacks* and A. J. Webb, Plant Diseases Division, Department of Scientific and Industrial Research, Auckland

(Received for publication, 2 May 1956)

Summary

In a field test, ferbam, zineb, thiram, dichlone, and copper oxychloride, prevented heavy establishment of celery leaf-spot (*Septoria apii* Chester).

Applications of ferbam, zineb, and copper oxychloride were almost equally effective at 7- and 14-day intervals; those of thiram and dichlone were more effective at the shorter intervals.

Introduction

In screening tests reported earlier (Jacks, 1955) a number of fungicides, e.g. ferbam, thiram, zineb, dichlone, maneb, etc., were more effective than others in preventing development of celery leaf-spot under glasshouse conditions. When plants sprayed with the above materials were washed (1 in. simulated rainfall), only ferbam, zineb, and dichlone provided residual protection.

This paper gives the results of a field test in which applications were carried out at 7- and 14-day intervals to ascertain the degree of control obtained under outdoor conditions.

Materials and Methods

Four-weeks-old celery seedlings of the variety Golden Self Blanching were planted on 28 April 1955 in rows 3 ft. apart with 1-ft. intervals between plants. Treatment replications consisting of rows 7 ft. long (6 plants per row) were randomized in five blocks, replicates being separated by paths 2 ft. in width.

The following materials were used:
Cop-ox (50% copper oxychloride) wettable powder;
Fermspray (70% ferbam) wettable powder;
Dithane D-14 (X/Y/Z i.e. X lb. of 19% nabam /Y lb. of zinc sulphate /Z lb. of hydrated lime) a tank-mix formulation of zineb;
Phygon XL (50% dichlone) wettable powder;
Thirospray (50% thiram) wettable powder.

Although only ferbam, zineb, and dichlone were resistant to simulated rainfall in glasshouse tests, copper oxychloride and thiram were included here to provide field evidence for two compounds commonly used by growers. Lack of facilities precluded the use of a larger number of compounds.

Spraying was commenced two weeks after planting. Applications were carried out at 7-day intervals (19 applications) and 14-day intervals (10 applications).

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The total rainfall from planting (28 April) until harvest (17 October) was 27.3 in.

Results were recorded on 6 and 22 September and 10 October 1955 by counting the total number of lesions on all leaflets of 18 leaf stalks (i.e. 3 per plant) from each replicate.

The means of these records are given in the Table 1.

TABLE 1. EFFECT OF SPRAY APPLICATION ON CONTROL OF CELERY LEAF-SPOT

Treatment	Concentration (lb./100 gal. water)	Lesions per 18 Leaf Stalks			
		Mean of 3 counts		Log (X + 1)	
		A*	B*	A	B
Check†	—	2877.0	2877.0	3.35	3.35
Cop-ox	5	30.7	91.3	1.38	1.65
Fermspray	4	14.0	23.3	0.83	1.22
Dithane D-14	2:1:0.5	20.7	33.0	1.05	1.25
Phygon XL	0.5	11.0	151.0	0.98	1.81
Thirospray	4	12.7	92.7	0.94	1.62
Difference required for significance at 1%:				0.63	0.73

* A, 7-day, B, 14-day intervals between spray applications.

† The same checks were used in both series of counts.

RESULTS

All materials, irrespective of application intervals, gave significant reduction of infection as compared with checks. Differences between fungicidal treatments were not significant.

Differences between results of applications carried out at 7- and 14-day intervals were not significant (s.d. at 1% = 0.65) for Fermspray, Dithane D-14, and Cop-ox. Phygon XL and Thirospray however, were significantly less effective at 14-day than at 7-day intervals.

This test confirmed neither the lower effectiveness of Cop-ox as assessed in screening tests nor the higher rain-resistance of Fermspray, Phygon XL, and Dithane D-14.

The significance of regular spray applications for prevention of the disease was proved by the greater economic value of treated plants as compared with checks.

CONCLUSIONS

Under conditions of these experiments copper oxychloride at 0.25% (Cop-ox at 0.5%), ferbam at 0.28% (Fermspray at 0.4%), tank-formulated zineb at 0.038% nabam + 0.1% zinc sulphate (Dithane

D-14 at 0.2%), dichlone at 0.025% (Phygon XL at 0.05%), and Thiram at 0.2% (Thirospray at 0.4%) effectively prevented heavy establishment of celery leaf-spot.

ACKNOWLEDGEMENT

Appreciation is expressed to Dr. H. R. Thompson of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for statistical analysis of results.

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THE INFLUENCE OF THE CALCIUM SULPHATE IN SUPERPHOSPHATE ON PHOSPHATE FIXATION IN SOILS

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(Received for publication, 13 April 1956)

Summary

1. A study has been made of the effect of calcium sulphate on the fixation in soil of phosphate added as monocalcium phosphate.

2. Eleven soils, some of a very high phosphate-fixing power have been used.

3. The presence of calcium sulphate reduced the amounts of water-soluble P_2O_5 consistently but temporarily, but increased "available" P_2O_5 as measured by various extractants.

Introduction

Superphosphate manufactured by the treatment of rock phosphate with sulphuric acid contains slightly more than half its weight of calcium sulphate. There has been a tendency to regard this gypsum as a useless filler, except in localities where the soil contains insufficient sulphur for optimum plant growth. The part played by the calcium sulphate in conditioning super is well known to the manufacturer. It seemed desirable to examine the possible effect of both the extra calcium ions provided and the sulphate ions, alone or in combination, on the fate of the phosphate when applied to soil. It has long been known that various salts influence the water solubility of calcium phosphates, for Liebig in 1863 showed that sodium chloride, sodium nitrate, and ammonium salts increase the water solubility though he did not demonstrate that this happened in the presence of soil. Dyer (1901) at Rothamsted carried out soil analyses which indicated that the sulphates of potassium, sodium, and magnesium had permitted greater penetration of the applied phosphate and that this phosphate was more easily soluble in 1% citric acid than in the absence of these salts. On the other hand Midgley (1931) reported that potassium sulphate and ammonium sulphate slightly decreased the penetration of superphosphate through a slightly acid silt loam. Hibbard (1935) found that calcium sulphate considerably depressed the water solubility of the phosphate that had been applied to four soils, but that other sulphates had little effect. Demolon, Boischot, and Lajon (1953) considered, using water solubility as the measure, that "the calcium sulphate present in ordinary superphosphate favours P_2O_5 fixation, at least temporarily, without causing a loss of assimilability".

It is apparent that the sulphate ion affects phoshate solubility but that this effect is considerably modified by the presence of other ions such as potassium, sodium, ammonium, etc., but apart from the work of Hibbard and Demolon *et al.*, referred to above, little attention has been given to the combination of calcium and sulphate ions, though it may be argued that in most of the work the phosphate has been added to the soil as one of the calcium phosphates and that calcium ions would be present in the soil solution.

Experimental

Preliminary trials were carried out with soils of four different types, viz. Taupo (pumice), Horotiu sandy loam, Manawatu silt loam, and Waikare clay loam. The air-dry soils were screened to pass a 1 mm. sieve. Ten pounds of soil were very throughly mixed with:

(1) 20 g. pure commercial monocalcium phosphate.

(2) Superphosphate fines containing the same total P_2O_5 as (1).

(3) 20 g. monocalcium phosphate previously intimately mixed with 20 g. calcium sulphate.

After thorough mixing, the soils were moistened carefully and mixed to give an even moisture content. The amounts of water used varied with the soils used and were adjusted to give a moist mixture without being sticky. The mixtures were kept in glazed earthenware jars closely covered to minimize moisture loss. Samples were taken at regular intervals for analysis. The following extractants were used:

(a) Water

(b) 1% citric acid

(c) 0.5 N acetic acid

(d) Truog solution

(e) Egner solution

In addition some samples were extracted with 0.5 M sodium bicarbonate at pH 8.5 (Olsen 1954), or with CO_2-saturated water.

Some difficulty was experienced in obtaining good duplicate analyses, with all extractants, where superphosphate was used. This is considered to be due to the particle size of the super being too large to allow of its thorough incorporation with so great an amount of soil. Grinding of super to produce particles approximating to those of the monocalcium phosphate used was not possible owing to the stickiness produced. However, the results obtained clearly showed that there was no important difference in behaviour between super and the mixture of monocalcium phosphate and calcium sulphate. Consequently in subsequent work super was not used.

Samples were analysed at weekly intervals in the early stages of the experiment and less frequently later. Apart from indicating the rapid rate of fixation of the added P_2O_5 in some soils, the later results show no changes in the relative behaviour of the various treatments. A typical set of analyses is given in Table 1.

The results in Table 1 show that the presence of calcium sulphate reduced the amount of water-soluble P_2O_5 in the four soils used, but with all the other extractants used there was a definite tendency for the calcium sulphate to increase the amount of P_2O_5 extracted.

To confirm and extend the results obtained a further experiment was carried out. In this the superphosphate addition was not made for the reason already given. The number of soils used was increased and include the following: Waiotira clay loam, Waikare clay loam, Papatoetoe silt loam, Taupo silty sand, Mangonui clay loam, Waitemata silt loam, Okaihau gravelly clay, and Taumata clay loam.

TABLE 1. PERCENTAGE P_2O_5 EXTRACTED BY VARIOUS EXTRACTANTS 21 DAYS AFTER MIXING

Extractant	Treatment	Soil type			
		Horotiu	Manawatu	Wa'kare	Taupo
Water	(1) Superphosphate	0.0005	0.018	0.009	0.007
	(2) Monocalc. phosphate	0.0014	0.031	0.017	0.009
	(3) „ plus $CaSO_4$	0.0006	0.018	0.009	0.007
1% citric acid	(1)	0.050	0.096	0.095	0.113
	(2)	0.035	0.082	0.079	0.103
	(3)	0.046	0.110	0.093	0.111
0.5 N acetic acid	(1)	0.009	0.059	0.035	0.032
	(2)	0.005	0.050	0.028	0.018
	(3)	0.008	0.064	0.033	0.025
Truog solution	(1)	0.0012	0.045	0.016	0.007
	(2)	0.0006	0.040	0.013	0.006
	(3)	0.0011	0.043	0.018	0.007
CO_2-water	(1)	0.0010	—	—	—
	(2)	0.0003	—	—	—
	(3)	0.0008	—	—	—
Egner solution	(1)	0.003	0.025	0.017	0.011
	(2)	0.002	0.024	0.010	0.005
	(3)	0.003	0.026	0.015	0.010
0.5 M sod. bicarbonate	(2)	—	—	—	—
	(1)	0.135	—	0.155	0.140
	(3)	0.141	—	0.174	0.150

The results of analyses made 1 week, 2 weeks, and 35 weeks after mixing are given in Table 2.

The figures given in Table 2 show a consistently higher content of available P_2O_5 in almost all soils where calcium sulphate was added. Although in the first few weeks this addition has lowered the water-soluble phosphate content, this difference has not been maintained, and after 35 weeks elapsed there was little difference between the treatments in this respect. It would appear that the calcium sulphate promotes the early fixation of the added phosphate in a water-insoluble form but does not result in greater total fixation. The figures for the

TABLE 2. PERCENTAGE P_2O_5 EXTRACTED AFTER 1, 2, AND 35 WEEKS

Soil	Treatment	Extract	Time		
			1 week	2 weeks	35 weeks
Waiotira clay loam	A*	(a) Water	0.020	0.014	0.008
		(b) Citric acid	0.128	0.128	0.076
		(c) Bicarbonate	0.189	0.189	—
	B†	(a)	0.009	0.006	0.006
		(b)	0.126	0.111	0.095
		(c)	0.201	0.187	—
Waikare clay loam	A	(a)	0.016	0.013	0.014
		(b)	0.128	0.132	0.088
		(c)	0.158	0.163	—
	B	(a)	0.008	0.008	0.009
		(b)	0.136	0.128	0.102
		(c)	0.175	0.164	—
Papatoetoe silt loam	A	(a)	0.007	0.007	0.003
		(b)	0.065	0.064	0.021
		(c)	0.171	0.170	—
	B	(a)	0.004	0.004	0.004
		(b)	0.067	0.075	0.035
		(c)	0.190	0.187	—
Flat Bush silt loam	A	(a)	0.013	0.015	0.003
		(b)	0.120	0.117	0.060
		(c)	0.172	0.176	—
	B	(a)	0.011	0.009	0.009
		(b)	0.144	0.130	0.083
		(c)	0.184	0.170	—
Horotiu sandy loam	A	(a)	0.006	0.006	0.001
		(b)	0.064	0.064	0.019
		(c)	0.135	0.127	—
	B	(a)	0.002	0.002	0.002
		(b)	0.076	0.073	0.040
		(c)	0.141	0.135	—
Taupo silty sand	A	(a)	0.008	0.008	0.003
		(b)	0.121	0.120	0.081
		(c)	0.140	0.136	—
	B	(a)	0.003	0.004	0.003
		(b)	0.141	0.137	0.103
		(c)	0.140	0.136	—
Mangonui clay loam	A	(a)	0.011	0.056	0.008
		(b)	0.040	0.052	0.085
		(c)	0.116	0.116	—
	B	(a)	0.002	0.002	0.002
		(b)	0.046	0.050	0.017
		(c)	0.122	0.106	—
Waitemata silt loam	A	(a)	0.025	0.023	0.010
		(b)	0.138	0.127	0.101
		(c)	0.187	0.187	—
	B	(a)	0.017	0.011	0.014
		(b)	0.149	0.137	0.126
		(c)	0.195	0.190	—
Okaihau gravelly clay	A	(a)	0.005	0.004	0.009
		(b)	0.030	0.030	trace
		(c)	0.118	0.126	—
	B	(a)	0.002	0.001	0.002
		(b)	0.040	0.044	0.011
		(c)	0.126	0.102	—
Taumata clay loam	A	(a)	0.004	0.002	0.002
		(b)	0.010	0.011	0.061
		(c)	0.137	0.136	—
	B	(a)	0.001	0.001	0.001
		(b)	0.013	0.011	0.087
		(c)	0.164	0.160	—

*A Monocalcium phosphate. †B Monocalcium phosphate plus $CaSO_4$.

extractions by 1% citric acid and by sodium bicarbonate indicate that in the presence of calcium sulphate the added phopshate is less firmly fixed than in its absence.

Discussion

The various extractants used in this investigation have all been used as a measure of the availablity of phosphate in the soil to plants. Admittedly there appears to be no method that can be relied on to give a sound guide regarding the necessity or advisability of making phosphate applications to a wide range of soils, but the fact that in these experiments all extractants, save distilled water and sodium bicarbonate, show higher "available" phosphate where calcium sulphate has been added, indicates that this material is not without effect on phosphate solubility and that such effect is not unfavourable.

The bicarbonate extraction figures indicate that no fixation of phosphate to an unavailable form takes place even in soils such as the Okaihau gravelly clay, which is known to be very highly fixing and on which heavy phosphate applications have only a short-lived effect on plant growth. Consequently this method cannot be regarded as being of use for the measurement of fixation of added phosphate. It does indicate differences in the natural phosphate of the soils in as much as the soils with the lowest bicarbonate-soluble phosphate are the least fertile.

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STUDIES ON METABOLIC DISORDERS IN DAIRY COWS

I. DIAGNOSTIC METHODS AND A SURVEY OF CLINICAL CASES OF KETOSIS, MILK FEVER, AND LACTATION TETANY IN NEW ZEALAND COWS

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Summary

An investigation of the metabolic disorders of dairy cows in the Waikato, Taranaki, and Manawatu districts has been made, in order to assess the importance of ketosis in the milk fever—grass staggers—ketosis syndrome.

The series studied is not entirely representative of any district or season, but is largely a selection of the atypical clinical conditions encountered in veterinary practice.

An attempt has been made to classify these according to the blood chemistry.

Uncomplicated acetonaemia is a relatively rare condition in this country, but a mild to moderate ketosis is frequently associated with grass staggers.

Introduction

The importance of the metabolic disorders in New Zealand may be gauged from the statement that one out of three visits made by veterinary practitioners in dairying districts is to a case of milk fever or grass staggers (Leslie 1945). It is therefore desirable that the variations in the biochemical picture which occur in these disorders should be understood, primarily that treatment may be correctly applied; fundamentally to elucidate their aetiology and aid in their prevention.

Soon after Little and Wright's (1925), Greig (1930) and Sjollema's (1930) work showed that milk fever was a hypocalcaemia and that grass staggers or lactation tetany was a hypomagnesaemia often associated with hypocalcaemia, these findings were confirmed for New Zealand conditions (Cunningham and Josland 1934). More recently it had been suggested, by field veterinarians here, that ketosis or acetonaemia in cows was a much more common and troublesome complaint than had hitherto been suspected.

A field survey was initiated by the writers to investigate this matter and also to ascertain if possible the aetiology of ketosis under New Zealand's grassland dairying conditions. As the investigation progressed, it became apparent that ketosis in its simple uncomplicated form was relatively rare, although a mild to moderate ketosis commonly occurred as a complication of, or accompaniment to, grass staggers. The study was then broadened to include cases of grass staggers and milk fever.

The term metabolic disease as used in this paper refers to the milk fever—grass staggers—ketosis complex in lactating cows. Transit tetany, although clinically and biochemically related to the metabolic diseases, is not included in this study, as no samples from cases of the disease were examined. Cases of so-called "paralysis", both before and

after calving in dairy cows, were seen on a few occasions, and, in those from which samples were taken, no biochemical abnormality was found. This disease cannot therefore be classed definitely among the metabolic diseases until more is known about it.

Milk fever or parturient paresis usually occurs soon after calving, and is characterized by neuro-muscular disturbances resulting in ataxia and paresis, and is accompanied by varying degrees of depression, extending finally to coma. Grass staggers or grass or lactation tetany usually affects relatively longer-calved cows, which show symptoms of hyperexcitability, hyperaesthesia, ataxia, tetanic convulsions and paresis, and, in later stages, depression and coma. Some writers use the term "atypical" grass staggers to describe cases of this type which occur within a few days of calving, and which may be regarded as being a combination of grass staggers and milk fever. Others use the term "atypical" milk fever for cases which show the typical milk-fever symptoms and blood picture, but which occur later than four days after calving. It is perhaps best to avoid the use of such terms and accept the fact that both milk fever and grass staggers vary widely in their time of occurrence. Ketosis or acetonaemia is by definition a biochemical abnormality, not a clinical condition, but the usual clinical accompaniments of a marked ketosis are anorexia, depression, decreased milk yield, loss of body condition, and occasionally hyperexcitability, hyperaesthesia, pica, and other neuroses.

Experimental

Design of the Investigation

The field investigation was designed to cover two aspects of the problem, firstly the biochemical character of the metabolic disorders and secondly their aetiology.

As many clinical cases as possible in herds in the Waikato district were visited in company with several different veterinarians in their normal daily rounds during August and September. A number of samples with accompanying clinical data have been provided by veterinarians in the Waikato, Manawatu, and Taranaki districts.

The series of cases studied are not a representative sample of those typically found in any district or season in New Zealand. It is to some extent a selection of the "atypical", "difficult", and "complicated" clinical conditions encountered by veterinarians in practice. A number of simple, uncomplicated cases are included chiefly for comparison.

Methods of Sampling

Samples of blood were drawn from the jugular vein into plain one-ounce bottles for serum calcium and magnesium estimations. In a few instances the serum was decanted into another bottle before despatch to the laboratory at Wallaceville, but usually, whole, clotted blood was sent. Serum was used for the acetone body estimations, as this was found to give almost the same results as oxalated blood, and had the advantage of avoiding the necessity of taking and despatching an extra bottle of blood. Inorganic phosphate and blood sugar were determined on 10-ml. samples of blood taken into bottles containing 22 mg. dried potassium oxalate and 100 mg. sodium fluoride. These samples were quite satisfactory provided they reached the laboratory within two or

three days of collection, and were not exposed to temperature above 25°c.

Urine samples were obtained in most cases from standing cows by gently massaging the cow's escutcheon from a few inches below up to the ventral commissure of the vulva, taking care not to touch the cow's tail, as this inhibits the micturition reflex. When this technique was unsuccessful in standing cows, and in cows which were recumbent, catheterization was performed, using a No. 12 Davol synthetic rubber catheter.

Methods of Analysis

Acetone Bodies—Rothera Test

The reagent used consisted of sodium nitro-prusside 1 g. finely ground and intimately mixed with ammonium sulphate, 99 g. The Rothera test was made by mixing about 1 g. reagent in a test tube with about 5 ml. urine or milk, adding about 1 ml. concentrated ammonium hydroxide, and assessing the resulting pink to purple colour after five minutes as negative, 1, 2, 3, or 4+. One version of this test using a flake of caustic soda as alkali gives erroneous results.

Where a strongly positive test was obtained, the sample was diluted 1 to 10 with water, and the test repeated. We have found it convenient to record the results of tests on 1/10 diluted samples as 10, 20, 30, and 40+.

Later, tablets containing a mixture of sodium nitro-prusside, anhydrous sodium carbonate, and ammonium sulphate were used. These provide a very convenient test in the field, two drops of test fluid being placed on the surface of the tablet and left for five to twenty minutes. In the presence of ketone bodies the surface of the tablet turns a red-violet colour, and the intensity can be graded up to 4+.

The Rothera test is positive only to acetone and acetoacetic acid, and not to β-hydroxybutyric acid which forms the main component of the acetone bodies.

Since acetoacetic acid, the most Rothera-sensitive of the acetone bodies, is unstable, the test will exhibit its greatest sensitivity on fresh samples.

It has been found that there is only an approximate relationship between the Rothera test and the quantitative determination of acetone bodies in the urine. A positive test on a urine sample does not necessarily indicate the presence of acetonaemia. A negative test on the urine, however, does indicate a normal blood ketone level (except in the very rare case involving kidney damage).

Sensitivity of the Rothera test. Aqueous solution containing 30 mg.% acetone will give a positive test. Urine samples containing total acetone-body levels of 40 mg.% as acetone will give a positive test. The normal range for cow urine is 3 to 50 mg.% total acetone bodies expressed as acetone. As a positive test is quite normal shortly after calving, it was found expedient to do the qualitative test on a 1:10 dilution of the sample. About calving a 4+ reaction on undiluted urine was not unusual. The sample diluted would give a reading of 1+ which would be reported as 10+.

Milk samples. It was found that a positive Rothera test on a milk sample definitely indicated a clinical ketosis, while a negative test did not necessarily exclude clinical ketonaemia or ketonuria. The mammary gland has been shown to utilise β-hydroxybutyric acid but not acetone and acetoacetic acid (Shaw 1941, 1942).

Saliva samples. The possibility of using saliva as a test fluid was investigated, but it was found to be much less sensitive to the Rothera test than milk.

Interference with the Rothera test. Occasionally urine samples were obtained showing a strongly positive Rothera test, while the corresponding blood ketone level was comparatively low or normal.

Various chemical compounds can give colour reactions under the conditions of the Rothera test. Certain phenols give red-brown colours and sulphydryl compounds give colours ranging from orange to red to purple and could be confused with a ketone body test. Since most sulphydryl compounds are normally excreted in the oxidized form, aberrant colours are unusual while the presence of ammonium sulphate in the reagent suppresses colour development. Unusual colours have not been observed during tests on the urine from dairy cows, but a fairly transient brilliant red colour was seen frequently in beef cattle urine and was probably due to a sulphydryl compound.

Acetone Bodies — Quantitative Test

Blood and urine acetone bodies were determined by means of a small-scale modification of the method of Van Slyke (1917) and Van Slyke and Fitz (1919), the amount of precipitate formed from the acetone present being determined iodimetrically. Milk samples were analysed by the method used for urine. Later a colorimetric method as used at Weybridge was employed (W. M. Allcroft, pers. comm.).

Blood Sugar

Fluoride-oxalated blood samples were used, and blood sugar was determined using Somogyi's (1945) reagent, combined with the colorimetric method of Nelson (1944).

Blood Inorganic Phosphate

Brigg's (1922) modification of the Bell and Doisy (1920) method was employed. The results are expressed as phosphate phosphorus.

Serum Calcium

The method of Clark and Collip (1925) was used.

Serum Magnesium

This was determined on the supernatant fluid obtained after the precipitation of calcium. The magnesium was precipitated as magnesium ammonium phosphate (Cunningham and Josland, 1934), and the phosphate determined by the method given above for inorganic phosphate.

Results

In presenting the results of the analyses of samples from cows showing clinical symptoms of metabolic disease, it is necessary to adopt

some method of classifying the results into groups of cases of one type, on either a clinical or a chemical basis. Neither method is entirely satisfactory, but the results may perhaps be more easily studied if they are classified on biochemical grounds. Accordingly, the cases have been grouped on the basis of the blood levels of calcium, magnesium, and acetone.

A biochemical grouping of results necessitates the fixing of a dividing line between normal and pathological values for the various blood constituents. There is a wide range of values in both normal and clinically abnormal cows for all three of the constituents considered here, and there is also a fair amount of overlapping between the ranges for normal and abnormal animals. Thus, any fine dividing line between normal and abnormal values will always place some normal animals in the abnormal range and some abnormal animals in the normal range.

The standards adopted for the characterization of the blood biochemical status in this paper are as follows:—

Normal serum calcium: 8.0 — 11.5 mg %
Normal serum magnesium: 1.5 — 3.3 mg %
Normal blood total acetone bodies: 0 — 16 mg %
Normal blood sugar: 40 — 70 mg %
Normal blood inorganic phosphate: 3 — 6 mg%

The minimal values given for normal serum calcium and magnesium are the upper limits for pathological hypocalcaemia and hypomagnesaemia respectively, and the maximal value for normal blood total acetone bodies is the lower limit of pathological acetonaemia. We believe these figures to have greater clinical significance than the range of values found in apparently clinically normal cows. The figures we have adopted as normal standards for sugar and phosphate are those found by Allcroft (1947a) in "a reasonably large number of animals which were in good condition and showed no symptoms of any clinical disorder".

The classified chemical results with their accompanying clinical data obtained on the cases studied from 1944 to 1948 are set out in Tables 1 to 5. In addition to cows showing obvious clinical symptoms, a few records are included appertaining to cows that were to all appearances clinically normal, but were running in herds in which there had recently been a more or less marked incidence of metabolic disease. These cows were picked out by the owners for examination and sampling mainly on suspicion of being a "bit off colour" or of "not doing very well"; in other words they may have just been subclinical but would not have been detected as abnormal by anyone but a close and regular observer.

The clinical notes have been kept down to a minimum in the tables, being a brief summary of the salient points regarding the case, and a note of any features of special significance. The breed of the cows studied was Jersey or predominantly Jersey unless otherwise stated. Where no mention of the number of calves produced to date by the cow is made, it can be presumed that the number is one less than the years of age, as the normal age at first calving for most New Zealand cows is two years. Full data are not given on some cows, either because they were not recorded, in the case of clinical data, or because they were unknown, as for example age of the cow. Chemical data are also

incomplete on some cases, as it was often obvious from the clinical condition that a full chemical study was not warranted.

As far as is known, no case here recorded had received any treatment prior to the samples being taken. In the few cases where a second set of samples was taken from a cow, no treatment had been given within several days of the second sampling, unless otherwise stated.

Cows that were "down" have been differentiated as far as possible into those that were "sitting", i.e. reclining normally, in sternal recumbency, and those that were "prostrate", or stretched flat out in lateral recumbency. Where no differentiation has been made, the condition has been described generally as "paresis". Unless otherwise stated, the cows were on their feet when sampled.

DISCUSSION

Hypocalcaemia — Hypermagnesaemia (Table 1). The fact that we found so few examples of this type of milk-fever blood picture may not be a true indication of its incidence. Numerous apparently typical milk-fever cases were seen but not sampled. Our main purpose was to study ketosis and grass staggers rather than milk fever. Milk-fever cases were only sampled when any unusual features were present, or when specially requested by the attendant veterinarian.

TABLE 1. HYPOCALCAEMIA — HYPERMAGNESAEMIA

Serum		Blood		Urine	Age (yr.)	Calved (Days)	Description of Case
Ca	Mg	T.a.b.* (mg%)	Gluc.	Rothera Test			
3.5	3.4					1	Prostrate, semicoma; slight tympanites.
5.0	3.4		109	–ve	9	2/24	Sitting, trying to stand: good milker, fairly fat.
7.5	4.0					1	Sitting, alert; paresis.

*Total acetone bodies

Hypocalcaemia — Normomagnesaemia (Table 2). Cases of this type were mostly those that would be described clinically as typical or simple milk fever, although some showed tetanic symptoms usually associated with the hypomagnesaemia of grass staggers. It is possible that such cows originally had a low serum magnesium, and that there had been a rise to within the normal range following the convulsive seizures. Cunningham (unpublished data, 1939) has found that convulsions provoked by several drugs cause a rise in serum magnesium. We have also observed a rise in serum magnesium following tetanic convulsions in a cow poisoned from eating mouldy Chewing's fescue seed cleanings. Some cows showed fairly strong urine Rothera reactions, but the blood figures do not indicate marked acetonaemia. Blood-sugar levels varied widely, with a tendency to be high. The one very low sugar value was of a cow that was dying, after being down overnight. She died shortly after treatment was commenced. Inorganic phosphate was subnormal in the few cases in which this constituent was determined.

TABLE 2. HYPOCALCAEMIA — NORMOMAGNESAEMIA

Serum		Blood			Urine	Age	Calved	Description of Case
Ca	Mg	T.a.b. (mg%)	Gluc.	Inorg. P.	Roth. Test	(yr.)	(Days)	
2.9	2.5						1	Sitting, alert, paresis.
3.1	2.7						1	Sitting, alert, paresis.
3.1	1.6		72	1.8		8	3	Prostr., semicoma; was sitting.
3.2	1.7						1	Staggering 1 hr. before; sitting.
3.3	3.0						2	Sitting, groaning; (tympanites).
3.3	2.7					5	1	Prostrate, semicoma; tympanites.
3.4	2.0						3	Prostrate, semicoma; tympanites.
3.7	1.8		60	1.1	20+	7	28	Prostrate, semicoma; fair condition.
3.8	2.6			1.0		5	2	Propped up, semicoma; fat, high producer; 3rd calf.
3.8	2.3						1	Sitting, alert, paresis.
3.9	2.3	4	15			5	3	Prostrate, coma, tympanites.
3.9	1.8						1	Ataxia.
4.0	3.0				10+	12	2	Swaying hindquarters; depressed.
4.0	2.9						1	Sitting, alert, paresis.
4.1	1.6						1	Staggering, stiffly.
4.2	2.7					10	6/24	Prostrate, spastic, been struggling.
4.2	1.5						1/24	Prostrate, semicoma.
4.3	2.5						3	Prostrate, semicoma.
4.6	2.0	4	87	1.3		4	3	Sitting, alternately quiet and excited. Calved; since grazed on fresh grass.
4.7	1.8						1/24	Ataxia just before & after calving.
4.9	2.4						1	Posterior paresis.
4.9	1.7						1	Sitting, slight depression.
4.9	1.6						4	Sitting, semicoma; change bare to flush pasture 12 hrs. before.
5.2	1.9						12/24	Prostrate, semicoma.
5.3	2.0						1/24	Sitting, depressed, down 1 hr. antepartum.
5.3	1.9						1	Sitting, trying to stand.
5.4	2.5					6	2	Unsteady gait; rather thin.
5.4	2.1						40	Opisthotonus, tetanic seizures, oestrus 2 days ago.
5.7	3.1	4	54			6	2	Ataxia; been down twice.
5.7	2.2	7	55	2.8	2+	6	2	Relapsed milk fever; gait normal at sampling, 30 hr. after 1st treatment.
5.7	1.8		190	1.2		9	1	Prostrate, semicoma, froth at mouth, tympanites; fat, dry last year.
5.7	1.5						2	Ataxia; had not been down.
6.1	2.5						1	Sitting, alert, paresis.
6.5	2.0						28	Tetanic seizures and semicoma.
6.8	2.9						40	Tetanic seizures and semicoma.
7.3	1.6				-ve		43	Prostrate, slightly dull; diarrhoea; oestrus previous night (Ayrshire).
7.4	2.6						1/24	Sitting; ataxia 1 hr. antepartum.
7.7	3.2					7	2	Slight ataxia, been down once.
7.7	1.8	8	41	2.3	20+	10	35	Sitting, very excited, trying to stand; heavy milker, fair condition, Urine T.a.b. 76mg%.
7.9	2.5					9	90	Sitting, slightly dull, paresis; good condition, heavy milker, 1/4 mastitis.
7.9	1.6	4			3+	9	1	Prostrate, semicoma; fat; constipated, tympanitic; on short grass (Aberdeen Angus).
8.1	2.7		57	4.3	10+	6	6	Sluggish gait, froth at mouth, dull, anorexia 4th calf (Ayrshire cross).

TABLE 3.—HYPOCALCAEMIA — HYPOMAGNESAEMIA

Serum		Blood			Urine	Age (yr.)	Calved (Days)	Description of Case
Ca	Mg	T.a.b.* (mg%)	Gluc.	Inorg. P	Roth. Test			
3.8	1.0					4	2	Prostrate, hyperaesthesia; slight tympanites.
3.9	0.3	2			-ve	4	1	Ataxia; tetanic seizure when approached (Friesian).
3.9	1.3		64	0.9		6	2	Prostrate, semicoma, struggling feebly.
4.0	0.9						10	Ataxia; tetanic seizure when handled.
4.1	1.4					4	3	Sitting, semicoma.
4.2	1.2					12	1	Sitting, semicoma.
4.4	0.8						1	Sitting, semicoma.
4.4	1.2						1	Sitting, semicoma
4.5	0.9						14	Ataxia, followed by prostration, semicoma.
4.5	1.3						3	Ataxia.
4.5	1.4	12	12		2+	5	30	Prostrate, semicoma, tympanites.
4.6	0.5	3	91	2.2	3+	8	3	Ataxia, slight hyperaesthesia, (Aberdeen).
4.7	0.8					8	50	Sitting, slight hyperaesthesia, oestrus 1 day.
4.7	1.3					5	2	Coma; down once before, twice after calving.
4.8	0.3	2	89	1.3	10+	6	2	Ataxia, muscular tremors, depressed.
4.9	1.0						1	Ataxia.
5.0	1.1	3	119	2.7		6	45	Sitting; tetanic seizure when handled; oestrus 2 days ago.
5.1	0.7	3	154	2.5	4+	8	8	Coma; poor condition tympanites.
5.1	1.1					5	20	Sitting, fairly alert; had milk fever earlier.
5.5	0.7	4				5	6	Sitting, fairly alert; had tetany last year.
5.6	1.1						1/24	Ataxia, hyperaesthesia, mild tetany.
5.9	1.1						1	Ataxia.
6.1	0.8	8	53		2+	5	38	Ataxia, hyperaesthesia; fell when handled; oestrus 1 day.
6.1	1.3					9	1	Sitting, fairly alert.
6.2	0.7	2	72		-ve	8	50	Ataxia, hyperaesthesia.
6.3	0.9				4+		26	Ataxia, tetanic seizure when handled, in oestrus.
6.4	1.0	3	167	3.4		5	41	Prostrate, hyperaesthesia, tetanic seizure.
6.6	0.4	2	54	2.8	4+	5	34	Ataxia, hyperaesthesia, falling repeatedly.
6.8	0.8					4	7	Ataxia, fell once; down and depressed.
6.8	1.1	3	107	3.0	1+	4	47	Sitting, slightly dull, trying to stand.
7.2	1.0	12	244	3.7	3+	7	28	Prostrate, semicoma; losing condition, hyperexcitable since calving.
7.3	0.6				-ve	4	46	Ataxia, hyperaesthesia, muscular tremors; down once 1 hr. pre-sampling.
7.7	0.8	1	85	7.8	-ve	3	13	Hyperaesthesia, milk yield decreased; feeding 2 calves (Aberdeen x Hereford).
7.9	0.7					7	6	Ataxia, hyperaesthesia; tetanic seizure 2 hr. pre-sampling.

* Total acetone bodies.

TABLE 3A. HYPOCALCAEMIA—HYPOMAGNESAEMIA PLUS KETOSIS

Serum		Blood			Urine	Age (Yr.)	Calved (Days)	Description of Case
Ca	Mg	T.a.b.* (mg%)	Gluc.	Inorg. P	Roth. Test			
3.7	0.4	24				4	9	Paresis, dull; tetanic seizure when treated.
5.4	0.3	18	132	1.7	40+	5	21	Paresis, dull, blepharospasm; good condition. (T.a.b. urine 594 mg%, milk 12 mg%, milk Rothera test 1+)*
6.0	0.7	17	149	1.0	20+	10	35	Ataxia, fell when driven; hyperaesthesia.
6.6	?	32	121		30+	9	1	Ataxia, poor condition, on poor feed (Haemolysis in serum;—Mg probably low).
(6.3	1.4	3	50		-ve	9	14)	Same cow as above; dull, anorexia, but milking fairly well. (Culled for tuberculosis).
6.9	0.6	16	62	4.7	2+	3	5	Prostrate, alert, eating hay; retained placenta; tetanic seizure 3 days ago (udder inflated); very heavy, fat, 1st calf Hereford. (Urine T.a.b. 26 mg%)*.
7.6	0.6	17	40	3.1	10+	5	24	Slightly nervous, losing condition since calving, milk yield decreased; (Urine T.a.b. 63 mg%, milk Rothera 2+)*.

* T.a.b. = Total acetone bodies.

Most of the cows in this group were mature, and the onset of symptoms occurred mainly within three days of calving. The longer-calved cows were mainly of the nervous tetanic type, although a few were quite dull and depressed. About three-quarters of the cows were recumbent, and of these, about equal numbers were sitting and prostrate.

Hypocalcaemia — Hypomagnesaemia alone or with Ketosis (Tables 3, 3A). Nervous symptoms were seen with greater frequency in these cases. Just under half of the total cases occurred within three days of calving. Similar symptoms were shown by cows recently calved and several weeks calved, both tetanic and comatose syndromes occurring early and later after calving. Ketosis was present in only about 15% of the total cows showing hypocalcaemia-hypomagnesaemia. Blood sugar tended to be high, with some very high levels even in the cases with ketosis. Some of these high figures may be accounted for by the fact that the samples were taken soon after the occurrence of a tetanic seizure, but this did not apply in all cases. Inorganic phosphate tended to be low in association with the lower calcium values, but some cows showed normal and even above-normal values. More second and third calvers were involved in these two groups than in the group with normal magnesium, although mature cows were also well represented. Approximately equal numbers of cows in this group were sitting, prostrate, and standing, but many of those standing went down when approached or handled.

TABLE 4.—NORMOCALCAEMIA — HYPOMAGNESAEMIA

Serum		Blood			Urine	Age (Yr.)	Calved (Days)	Description of Case
Ca	Mg	T.a.b.* (mg%)	Gluc.	Inorg. P	Roth. Test			
8.4	0.4					3	23	Prostrate, hyperaesthesia, tetanic seizure 2 hrs. pre-sampling.
9.7	0.4	12	46	3.2	30+	3	24	"Not doing well"; losing condition, milk decreased since milk fever attack 3 weeks ago. (Urine T.a.b. 134mg%, milk Rothera -ve) *.
8.4	0.5				2+		10	Tetanic seizure (Aberdeen Angus).
8.9	0.5	6	61	3.2	3+	6	38	Ataxia, head held high, hyperaesthesia; poor condition, milking poorly, anorexia. (Urine T.a.b. 10mg%, milk Rothera -ve) *.
8.7	0.6	3	63	5.4	2+	4	20	Excited, fell when handled; losing condition, feeding 2 calves (Beef Shorthorn).
10.1	0.6						29	Ataxia, hyperaesthesia; fair condition.
8.7	0.7	2	108	5.6	1+	3	107	Tetanic seizure, followed by paresis and coma; in good condition, on short pasture.
8.0	0.8				-ve		4	Sitting, hyperaesthesia; tetanic seizure when handled.
9.7	0.8	3			4+	8	35	Excited, aggressive, staggery, diarrhoea, hyperaesthesia; fell when caught.
9.7	0.9	9			1+	3	12	Ataxia; tetanic seizure and paresis; poor producer, good condition (Ayrshire).
9.2	0.6	7	70		1+	3	42	Same cow as above; nervous and trembly since 1st attack; tetanic seizure in bail; sampled 1 minute after convulsions began.
8.2	0.9	3				5	12	Prostrate, semicoma; tetanic seizure.
10.0	0.9					4	42	Ataxia, hyperaesthesia, tremors; anorexia; losing condition last two weeks.
10.5	0.9	2	94	5.2	3+	3	28	Tetanic seizure, paresis (Aberdeen Angus).
8.0	1.0					3	1	Prostrate, semicoma (milking Shorthorn).
10.5	1.0	6	43	3.2	2+	4	17	"Slightly off colour"; fair producer, good condition (Ayrshire).
8.9	1.2					4	21	Standing, trembling, hyperaesthesia; tetanic seizure 1 hr. pre-sampling.
9.7	1.2				-ve	4	150	Staggering, falling repeatedly, trembling, hyperaesthesia (Aberdeen Angus).
8.9	1.3	3			1+	11	40	Ataxia, hyperaesthesia; later paresis, becoming comatose.
10.6	1.4						12/24	Sitting, eating, paresis only slight.
10.6	1.4					3	30	Ataxia, hyperaesthesia, anorexia; in oestrus 1 day.

* Total acetone bodies.

TABLE 4A. NORMOCALCAEMIA — HYPOMAGNESAEMIA PLUS KETOSIS

Serum		Blood			Urine			
Ca	Mg	T.a.b. (mg%)	Gluc.	Inorg. P	Roth. Test	Age (Yr.)	Calved (Days)	Descrption of Case
8.8	0.6	20	56	4.7		4	25	Prostrate, paresis, hyperaesthesia; losing condition.
9.9	0.6	16	54	2.5	30+	4	20	Gait normal, slight hyperaesthesia; tetanic seizure followed by ataxia few hours ago. (Urine T.a.b. 425 mg%) *.
8.1	0.7				30+	6	30	Ataxia, hyperaesthesia; very poor feed.
8.2	0.7	31	35	1.5	20+	5	20	Dull, not eating; excited and trembling, nearly fell when bled and dosed.
10.2	0.9	16	53	3	30+	5	40	Ataxia, hyperaesthesia, tremors.
10.3	0.9	43	340	4.6	30+	5	15	Going down on knees in paddock last few days; appeared normal; good milker, fat condition (Ayrshire). (Urine T.a.b. 290mg%) *.
9.9	1.1	45	36	3.3	20+	4	27	Dull, losing condition and milk; (Urine T.a.b. 180mg%, milk Rothera 1+) *.
9.0	1.2	25				5	16	Paresis, hyperaesthesia; ataxia (see next sample).
(5.1	0.6	11)						20 hrs. after treatment; semi comatose.
9.7	1.3	19	69		1+	4	44	Alternately moping and excited; gait normal; losing appetite, condition and milk, over last day or two.

Normocalcaemia — Hypomagnesaemia, alone or with Ketosis (Tables 4, 4A). These cows were almost all past the second week of lactation, two of them three and five months after calving. Over 80% of these cows were still on their feet, though a few fell when approached or handled. Nervous symptoms were commonest in these two classes, many showing typical tetanic seizures. Blood sugar also tended to be high, the highest value occurring in an Ayrshire cow with ketosis (6th cow in Table 4A). Inorganic phosphate was normal in most cases. About one-third of the cows with low magnesium and normal calcium had ketosis, as compared with one-sixth in the cases where calcium was also low.

Normocalcaemia — Normomagnesaemia — Ketosis (Table 5). Calcium and magnesium were not determined on many of these samples, but from the general clinical description of the cases it is fairly safe to assume that their calcium and magnesium figures were normal. These samples were taken by veterinarians in Taranaki in 1944. The samples in which calcium, magnesium, glucose, and phosphate were determined were from Waikato and the Wallaceville neighbourhood. Of the four local cases, one was secondary to lead poisoning, another was secondary to pyometra following retained placenta, and the third was the only case of typical "nervous" uncomplicated ketosis in our series. The others were all of the so-called "digestive" type: dull, losing condition, and

TABLE 5. NORMOCALCAEMIA — NORMOMAGNESAEMIA PLUS KETOSIS

Serum		Blood			Urine			Milk			Age (Yrs.)	Calved (Days)	Description of Case
Ca	Mg	T.a.b.* (mg%)	Gluc.	P	Roth. Test	A + A†	T.a.b.	Roth. Test	A + A†	T.a.b.*			
		146			10+			1+					*Individual clinical notes not recorded in several cases.* Where no records are given the samples were from cows showing typical "digestive" syndromes: dull, losing appetite, condition, and milk yield. Acetone detected on breath of several cases, and flavour in milk of some of those. Most cases occurred from 4 to 6 weeks after calving. No nervous symptoms were reported or observed. No cows were "down", or showing incoordination in walking.
		120			30+	232	898						
		107			40+	215	857	3+	22				
		92			40+		117	2+	15				
		90			40+	146	548	2+	29				
		81			30+	174	582	2+	22	42			
		79			40+	113	469	3+	32				
10.2	2.6	79	25	3.9	30+	122	470	2+	15	25	5	14	Milk yield suddenly almost suppressed; had staggered coming in for milking.
9.9	3.2	77	20		20+		264	3+		46	6	13	Losing condition, milk, appetite; coat dull and staring; pyometra present.
		60			30+	167	498	?+	4				No individual records. See general description above.
10.0	2.1	60			40+		500	3+		41	8	200	Losing condition, milk and appetite last 2–3 weeks; thin, anaemic, constipated.
10.1	2.5	51	42		40+		420	1+		12	6	26	Losing condition, milk and appetite last week; coat dry and rough; slight muscular tremors.

10.1	2.5	47			40+		1420	1+		14	3	92	Losing condition, milk and appetite last 2 days, coat dry and rough; lying down a lot. (Lead poisoning suspected and confirmed after death three days later).
		47			40+	93	257	2+	13				No records.
		38			30+	195	427	?+	4				No records.
		37			30+	139	381	?+	1				No records.
		22			20+	53	185	—	0.4				No records.
9.5	2.3	21	41	4.2	4+		41	—					Slightly "off colour", decreased milk yield last few days; very good condition.
10.2	2.9	17			40+		345	1+		4	4	13	Slight ataxia; marked excitement, frothing at mouth when handled; neck stiff and head raised; losing condition, milking very well on poor, short pasture; no hay fed since calving. No odour of acetone detected on breath.
8.9	2.0	55			40+							44	Same cow as above. Third attack. Was nervous at 2nd attack, but not now. Responded to carbohydrate therapy at previous attacks. Now in poor condition, not eating, milk yield reduced 50%, depressed, but coat and eye bright. (Relapsed twice more in following month, recovering each time when taken to good pasture.)

All values are expressed as mg%.

† A + A = Acetone + aceto-acetic acid.

* T.a.b. = Total acetone bodies.

with depressed milk yield and appetite. None, except the lead-poisoning case died or even became paretic as a result of the disease.

Those simple ketosis cases of which we have full individual records were all past the twelfth day of lactation. The youngest cow whose age is known was the nervous case, a four-year-old. The cow that was furthest from calving (7 months) developed the condition in mid summer. All the others occurred in late winter or spring, when pasture growth was young and fresh, but fairly scanty in amount.

Blood-sugar levels were normal or low and inorganic phosphate normal in the few cases examined.

TABLE 6. METABOLIC DISEASES IN DAIRY COWS

Blood Picture as in Table—	Low Ca Normal or High Mg 1 & 2	Low Ca Low Mg ± Ketosis 3 & 3A	Normal Ca Low Mg ± Ketosis 4 & 4A	Normal Ca Normal Mg Ketosis 5
Disease Name	Milk fever (Some ? grass staggers)*	Grass Staggers. ± Ketosis.	Grass Staggers. ± Ketosis.	Ketosis (Simple).
Time After Calving	Mostly 1–3 days; (Some up to 3 months)	About equally common 1–3 days, 4–21 days, 22–50 days.	Mostly 10–40 days; (Some earlier, some later).	Mostly 10–40 days; Some later.
Attitude	About equal numbers prostrate, sitting, staggering.	As in previous column.	Mostly staggering; some prostrate, some sitting.	Mostly walking steadily; some stagger slightly.
General symptoms	Paresis, coma (some excitement, tetanic seizure).	Mostly excited, "touchy" trembling, twitching, convulsing; some paresis, coma.	As in previous column; but less paresis, coma.	Anorexia, depression, agalactia, wasting; some nervous symptoms.
Urine Rothera reaction	-ve to 20+	-ve to 10+ without ketosis; (2+) 20+ to 40+ with ketosis.	-ve to 3+ (30+) without ketosis; (1+) 20+ to 30+ with ketosis.	(10+) 20+ to 40+ (Milk -ve to 3+).
Inorg. P	Low	Low with low Ca but some normal and above normal.	Normal in most cases.	Normal

* Cases showing tetanic convulsions.

A summary of the data in tables 1 to 5 as set out in table 6 shows in a general way the correlation between the chemical classification and clinical findings in the series of cases studied. Table 6 shows that an accurate diagnosis of the blood chemical picture could not be made in many cases from the clinical data given. However, by taking into acount all the relevant data on a given case, the accuracy of diagnosis could be increased beyond that obtainable from the broad general facts considered here.

What other information is relevant or helpful in clarifying the diagnosis in obscure cases is beyond the purpose of this paper to consider in detail. Very few clinical syndromes alone are sufficient to identify any individual metabolic disorder.

Much information of value may be obtained from the past history, feeding conditions, and general environmental background of the cow in many cases. Other data that may be helpful in some cases are age, milk-production records, condition at calving and at the time of the disease attack, temperament, obstetrical history, and the stage of the sexual cycle.

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MILK PRODUCTION IN FIRST-CALF HEIFERS FOLLOWING GROWTH-HORMONE THERAPY

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Summary

1. Nine sets of identical-twin, first-calf heifers were split into two identically fed and managed groups. One group received 50 mg. of growth hormone daily for approximately 14 days prior to calving and for a further 14 days thereafter. The other group received saline only during this period.
2. During the period of therapy and for some seven days thereafter the milk production of the growth-hormone group was appreciably greater than that of the controls. Fat test was unaltered.
3. No appreciable carryover effects of this increased milk production into the main lactation period were apparent.

Introduction

Recently it has been established that growth hormone, when administered to lactating cattle, is capable of increasing milk production[1, 2, 3]. This increased productive capacity is apparently not associated with any change in the levels of major blood or milk constituents, and, surprisingly, appears to be accompanied by a higher net efficiency of milk production[1]. The only possible clue to the mechanism involved that Brumby and Hancock obtained in a recent experiment was a possible direct effect on mammary development[1]. Such a concept received considerable reinforcement when Flux (unpublished data) demonstrated an increase in both mammary and uterine growth in hypophysectomized mice subject to growth and thyrotropic hormone therapy and further when Chung[4] demonstrated that growth hormone administered to first-calving heifers for some 24 days prior to calving greatly increased subsequent milk production. This latter observation was of appreciable significance in that, if indicative of a general situation, it may offer a practical means of utilizing the marked galactopoetic properties of growth hormone.

Some nine sets of first-calving heifers were therefore treated with growth hormone about the time of parturition, and their subsequent lactation performance was recorded.

Experimental

Nine sets of twins were randomly divided into the two groups indicated in Table 1. The expected calving date for each animal, computed as 283 days after conception, is tabulated together with the actual calving date. During the fourteen days preceding the expected calving date each animal in the growth hormone group received 50 mg. of growth hormone (Armour Lot No. M 308) dissolved in 4.5 cc. of physiological saline and injected subcutaneously, whilst the animals in the control group received 4.5 cc. saline only. Following the actual calving date the same treatments were continued for a further 14 days. Apart from the hormone therapy all animals were treated identically and run together as one herd of cattle grazing pasture *ad lib.* and provided

TABLE 1.—EXPERIMENTAL GROUPS, EXPECTED AND ACTUAL CALVING DATE

Growth-hormone Group			Control Group		
Twin No.	Expected Calving Date	Actual Calving Date	Twin No.	Expected Calving Date	Actual Calving Date
179	19.7.55	24.7.55	180	3.9.55	31.8.55
333	15.7.55	18.7.55	334	24.7.55	29.7.55
502	31.7.55	29.7.55	501	18.7.55	14.7.55
515	21.7.55	23.7.55	516	11.7.55	13.7.55
528	11.7.55	14.7.55	527	27.7.55	25.7.55
533	25.7.55	26.7.55	534	30.7.55	7.8.55
540	23.7.55	23.7.55	539	20.7.55	25.7.55
549	6.8.55	13.8.55	550	17.7.55	10.8.55
5115	13.7.55	16.7.55	5116	24.7.55	1.8.55

with six pounds of a mixed dairy ration each day after calving. The recording of daily milk yields commenced four days after calving. During the preceding three days the calf ran with the cow and suckled freely, whilst once each day the cow's udder was evacuated by normal milking. Three times each week during the first three weeks of lactation and once weekly thereafter a composite a.m.-p.m. milk sample was tested for fat content.

RESULTS

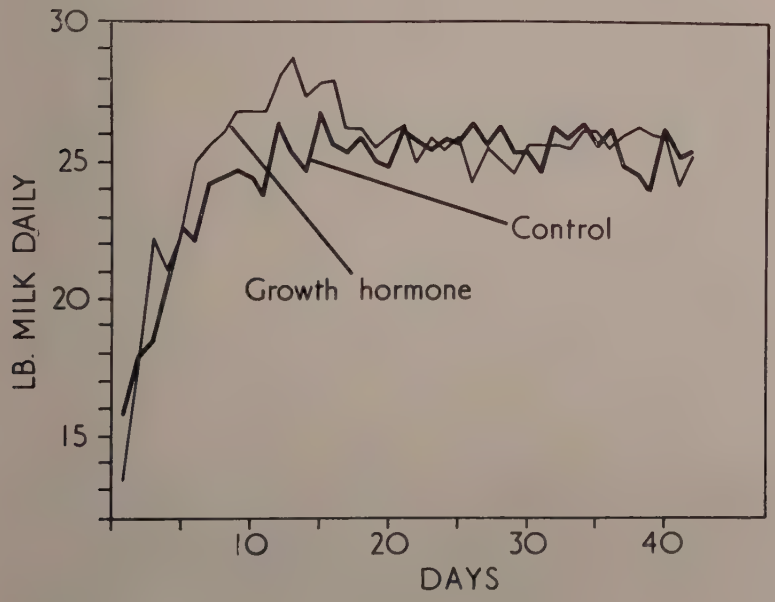

Fig. 1.—Average daily milk weights.

Tables 2, 3, and 4 list the seven-day-total milk weights, fat test, and fat yield respectively for each cow during the first six weeks of lactation, whilst Fig. 1 presents a daily plot of group-average milk weights throughout the same period.

TABLE 2. TOTAL MILK PRODUCTION IN LB. THROUGHOUT CONSECUTIVE SEVEN-DAY PERIODS

Twin No.	1st 7 days	2nd 7 days	3rd 7 days	4th 7 days	5th 7 days	6th 7 days
Growth-hormone Group						
179	263.7	278.2	228.4	212.4	211.2	200.5
333	221.3	272.6	264.6	217.0	202.5	201.9
502	81.0	125.4	119.8	112.3	125.6	127.4
515	178.6	215.3	196.4	187.0	196.1	195.0
528	185.7	239.9	236.9	234.0	227.9	232.1
533	92.5	143.2	147.4	153.1	159.0	153.2
540	92.7	135.2	143.0	127.2	135.9	144.8
549	83.5	133.2	172.3	185.5	184.3	193.8
5115	145.3	173.1	165.9	164.4	166.9	168.1
Average	149.4	190.7	186.1	177.0	173.9	179.6
Control Group						
180	221.8	242.0	247.8	235.5	201.7	211.1
334	217.4	250.0	241.4	243.1	240.4	204.8
501	98.3	111.5	113.4	117.5	117.1	101.9
516	150.3	169.1	175.7	173.8	179.9	180.5
527	174.3	208.0	216.1	227.6	222.0	217.3
534	136.4	168.6	183.3	199.1	194.8	204.1
539	85.7	137.1	134.4	126.3	138.1	145.6
550	58.6	124.2	139.3	126.5	134.6	141.7
5116	111.6	153.7	165.4	178.0	184.9	180.5
Average	139.4	173.8	179.6	180.8	179.3	176.4

It is apparent that, during the period of growth-hormone therapy, daily milk production was appreciably increased, there being, however, little carryover effect. Thus milk production in the two groups was again even some three weeks after calving. Growth-hormone therapy prior to parturition appears to have been of little consequence in that four days after calving when the first milk weights were recorded the

difference in production was slight, being increased, however, by subsequent therapy. The fat test was not apparently altered by the experimental treatment imposed.

TABLE 3. AVERAGE FAT TEST THROUGHOUT CONSECUTIVE SEVEN-DAY PERIODS

Twin No.	1st 7 days	2nd 7 days	3rd 7 days	4th 7 days	5th 7 days	6th 7 days
Growth-hormone Group						
179	3.7	4.0	4.5	4.7	4.4	3.6
333	3.7	3.8	3.2	3.0	2.6	3.4
502	4.4	4.9	5.8	3.6	4.1	3.4
515	3.9	4.8	4.5	5.1	5.1	4.5
528	4.3	4.1	3.5	4.2	4.5	4.0
533	3.8	4.0	2.7	4.8	3.4	4.2
540	4.5	4.7	4.8	5.1	6.1	5.1
549	6.4	5.1	3.9	4.0	4.0	4.0
5115	4.6	4.1	4.1	3.9	7.4	4.2
Average	4.4	4.4	4.1	4.3	4.6	4.0
Control Group						
180	4.0	3.9	3.9	4.4	3.7	4.4
334	4.6	5.2	3.5	3.9	4.6	4.4
501	5.3	3.6	4.3	3.7	3.1	2.8
516	4.7	3.7	4.4	4.3	4.7	5.2
527	3.4	4.3	4.3	3.6	4.1	4.4
534	3.7	3.4	3.6	3.8	3.8	3.9
539	3.5	4.7	4.5	5.7	4.8	4.8
550	4.5	3.8	4.5	3.8	4.3	4.8
5116	4.8	3.7	3.9	5.0	4.6	4.6
Average	4.3	4.0	4.1	4.2	4.2	4.4

DISCUSSION

The results presented do little to refute the suggestion that the effects of growth hormone on lactation in the cow may be mediated through an influence on mammary growth. However, they certainly indicate that a short-term period of therapy about the time of parturition, and at a time when the mammary gland is undergoing a marked increase in activity, is likely to have residual effect. It may be that a similar

TABLE 4. BUTTERFAT YIELD THROUGHOUT CONSECUTIVE SEVEN-DAY PERIODS

Twin No.	1st 7 days	2nd 7 days	3rd 7 days	4th 7 days	5th 7 days	6th 7 days
Growth-hormone Group						
179	9.8	11.1	10.3	10.0	9.3	7.2
333	8.2	10.4	8.5	6.5	5.3	6.9
502	3.6	6.1	6.9	4.0	5.1	4.3
515	7.0	10.3	8.8	9.5	10.0	8.8
528	8.0	7.8	8.3	9.8	10.3	9.3
533	3.5	5.7	4.0	7.3	5.4	6.4
540	4.2	6.4	6.9	6.5	8.3	7.4
549	5.3	6.8	6.7	7.4	7.4	7.8
5115	6.7	7.1	6.8	6.4	12.4	7.1
Average	6.3	8.0	7.5	7.5	8.2	7.2
Control Group						
180	8.9	9.4	9.7	10.4	7.5	9.3
334	10.0	13.0	8.4	9.5	10.1	9.0
501	5.2	4.0	4.9	4.3	3.6	2.9
516	7.1	6.3	7.7	7.5	8.5	9.4
527	5.9	8.9	9.3	8.2	9.1	9.6
534	5.0	5.7	6.6	7.6	7.4	8.0
539	3.0	6.4	6.0	7.2	6.6	7.0
550	2.6	4.7	6.3	4.8	5.8	6.8
5116	5.4	5.7	6.5	8.9	8.5	8.3
Average	5.9	7.1	7.3	7.6	7.5	7.8

type of therapy instituted in the early phase of pregnancy when the mammary gland exhibits maximum growth of ducts would be of greater significance in the subsequent lactation, but this point remains to be clarified.

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FIELD TESTS FOR CONTROL OF BROAD-BEAN RUST

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(Received for publication, 30 April 1956)

Summary

In field tests carried out over three seasons to assess the value of sprays in controlling broad-bean rust (*Uromyces fabae* (Pers.) de Bary) the following results were obtained.

The outstanding material was Dithane Z 78 (Zineb) closely followed by Fuclasin Ultra (Ziram).

Other materials gave similar control but for shorter periods, Manzate, Thirospray and Spergon being the best of these.

Introduction

Promising materials for control of broad-bean rust were selected from screening tests, results of which have been reported by Jacks (1954). Field tests reported in this paper were carried out over three seasons to measure the effect of these materials under outdoor conditions, and to determine the intervals necessary between applications.

Materials and Experimental Methods

Broad bean seeds of the variety Yates Early Longpod were sown in rows 1 ft. apart. There were four rows of 5 ft. length in each plot and plots were separated by paths 2 ft. in width. There were five replications for each treatment, plots being randomized within blocks.

The following materials were used:

Lime sulphur (15% polysulphides) liquid;
Colsul 40 (40% colloidal sulphur) paste;
Cosan (70% sulphur) wettable powder;
Aasulpha Supra (75% sulphur) wettable powder;
Phygon XL (50% dichlone) wettable powder;
Spergon w.p. (48% chloranil)wettable powder;
Flit 406 (50% captan) wettable powder;
Dithane Z 78 (65% zineb) wettable powder;
Manzate (70% maneb) wettable powder;
Fuclasin Ultra (70% ziram) wettable powder;
Thirospray (50% thiram) wettable powder; and
PEPS (80% polyethylene polysulphide latex), wettable powder.

PEPS was added to one series of applications to ascertain the effect of a sticker on resistance to weathering.

Spraying was commenced when plants were 1 ft. high the amounts applied varying according to size of plants (from $\frac{1}{4}$ to 1 gal. per replicate).

Applications were carried out in 4 series viz. at 7-day intervals (12 applications), at 10-day intervals (8 applications), at 14-day intervals (6 applications) and at 21-day intervals (4 applications).

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Seed was sown in April of each of the three years in which trials were carried out and records were taken in September.

Records were made at completion of each trial period by counting the total number of rust lesions per leaf on 10 leaves taken from each plant. Two counts were made at 10-day intervals (one early in September and the other in mid September) and the means of these counts are given in the Table.

TABLE 1. MEAN EFFECT OF SPRAY APPLICATIONS, OVER THREE SEASONS, ON CONTROL OF BROAD-BEAN RUST

Treatment	Concentrations in lb. per 100 gal. water	Lesions per Leaf No. of days between applications							
		7	10	14	21	7	10	14	21
		Mean number of lesions				Log. (x + 1)			
Check—water	—	75.2	62.9	79.6	67.8	1.28	1.48	1.32	1.51
Lime sulphur	8.11	39.7	34.9	44.2	48.3	0.68	0.94	0.94	0.99
Lime sulphur + Colsul 40	6.18 + 2	19.0	33.3	37.9	54.0	0.49	0.87	0.85	1.15
Cosan	4	26.0	21.9	33.0	28.2	0.84	0.69	0.96	0.97
Aasulpha Supra	375	8.2	5.2	19.5	19.6	0.64	0.52	0.79	0.67
Phygon XL	1	16.5	25.7	23.9	28.5	0.48	0.67	0.70	0.96
Spergon w.p.	1	0.6	6.9	18.1	69.6	0.18	0.31	0.75	1.16
Flit 406	2	23.6	20.6	20.5	25.8	0.55	0.66	0.64	0.93
Dithane Z 78	2	0.0	0.2	0.0	1.6	0.00	0.04	0.00	0.26
Manzate	0.5	0.1	0.3	0.3	15.3	0.01	0.12	0.08	0.64
Fuclasin Ultra	2	0.1	0.8	0.6	5.8	0.01	0.19	0.12	0.56
Thirospray	2	0.1	1.2	12.7	17.0	0.04	0.28	0.40	0.77
Sig. diff. at 1%:						0.27	0.33	0.29	0.36

RESULTS

When comparisons were made with the most effective material in each series of applications the following treatments were found to give equivalent control:

1. Applications carried out at 7- and 10-day intervals: Dithane Z 78 at 2 lb. (zineb at 0.13%); Manzate at 0.5 lb. (maneb at 0.035%); Fuclasin Ultra at 2 lb. (ziram at 0.14%); Thirospray at 2 lb. (thiram at 0.1%); and Spergon w.p. at 1 lb. in 100 gallons of water (chloranil at 0.048%).

2. Applications carried out at 14-day intervals: Dithane Z 78 at 2 lb. per 100 gallons of water (zineb at 0.13%); Manzate at 0.5 lb. (maneb at 0.035%); and Fuclasin Ultra at 2 lb. (ziram at 0.14%).

3. In applications carried out at 21-day intervals: Dithane Z 78 at 2 lb. per 100 gallons of water (zineb at 0.13%) was the outstanding treatment, although Fuclasin Ultra at 2 lb. (ziram at 0.14%) was not significantly different.

The addition of PEPS at ½ lb. per 100 gallons of water (polyethylene polysulphide at 0.04%) did not increase the effectiveness of any material.

Conclusions

Applications of zineb or ziram (at 0.14%) carried out at 21-day intervals gave satisfactory control of broad-bean rust.

Acknowledgement

The authors thank Dr. H. R. Thompson of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for carrying out statistical analysis of results.

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ROOT DEVELOPMENT IN SOME COMMON NEW ZEALAND PASTURE PLANTS

IX. THE ROOT REPLACEMENT PATTERN IN PERENNIAL RYEGRASS (*Lolium perenne*)

By W. A. Jacques, Massey Agricultural College, Palmerston North

(Received for publication, 16 February 1956)

Summary

An account is given of the root deterioration and root replacement patterns of perennial ryegrass. An idealized diagram is given showing the periods at which they occur. They fit into an annual pattern of replacement.

Introduction

It is generally appreciated that the nodal roots of grasses are periodically replaced by new root growth. Longevity studies by various workers have shown that there is a considerable amount of inter-specific variation in the length of life of roots and consequently in their rate of replacement.

Studies have been conducted on perennial ryegrass and other species at this College with a view to determining the replacement pattern. This paper is based on data acquired in pot trials and from observations made following excavation of the root systems to study their pattern and behaviour under natural growth conditions.

Review of Literature

Troughton (1955) has pointed out that an adequate technique has not yet been devised to enable a functional root to be distinguished from a root that is of no value to the plant, i.e. non-functional and presumably dead. The criterion commonly used has been the decay of the root, but caution is needed in its use as the cortex of a root may decay leaving a functional stele. Again, a root may remain undecayed long after it has become non-functional.

Cepikova (1942) reported that the length of life of roots varies as between roots of the same plant and between plants of different species. Stuckey (1941) divided the species studied into those with "annual" and those with perennial root systems. Those of perennial ryegrass she classified as "annual". Sprague (1933) considered that half of the root systems of both *Poa pratensis* and *Agrostis tenuis* were regenerated each spring, but Stuckey regards the latter as probably "annual". Stoddart (1935) and Weaver and Zink (1946) used a banding technique and showed that the length of life of roots of the same species was not uniform and the interspecific differences were considerable. Weaver and Zink showed that longevity was affected by defoliation. Pavlychenko (1942) and Stevenson and White (1941) noted that roots of *Agropyron pauciflorum* were short-lived, while Jefferies (1916) considers that roots of *Molinia coerulea* continue to function for three years. Jacques and Schwass (1956) working with three grasses give hypothetical figures for the annual root replacement of Italian ryegrass at 100%, perennial ryegrass at 68%, and tall fescue at 60%. They stress however that these figures are based on factors

that can be modified by environmental and treatment changes, and so can vary within wide limits.

THE PATTERN OF ROOT REPLACEMENT

Whatever may be the percentage figure for the annual replacement of roots, there is in perennial ryegrass a replacement pattern which is determined primarily by climatic conditions.

When seed is sown in March, there is an increasing amount of root formed (both in volume and depth of penetration) as the plant ages. The amount formed is complementary to the soil type, the treatment of the tops, and the general environmental conditions. Roots of perennial ryegrass sown in March and grown on a deep well-drained silt loam in Palmerston North have penetrated to below 6ft. by the end of December of the same year.

Until October, new root initiation is rapid (Jacques and Edmond 1952) but, thereafter, throughout late spring and summer relatively few new roots are formed, so that root development at this time is centred on the deep-seated roots. The plants go through the summer on the roots formed earlier in the season. When the plants are a year old there is a renewal of a similar pattern of growth, i.e. from March to October new roots are initiated and increase in number, and from October to March there is an increase in depth only.

THE PATTERN OF DETERIORATION

Root deterioration becomes apparent first (usually) in October. This is evidenced by a softening of the cortex preparatory to its sloughing off, thus exposing the stele. Numerous workers (as reported by Troughton 1955) have shown that food materials are stored in the roots during periods of slow herbage growth. These reserves are drawn on when rapid growth of the top takes place. In New Zealand, such a period occurs in October–November when there is not only a sudden increase in the amount of leaf produced, but there is a call on food reserves to satisfy the requirements of the flower-head primordia. As the cortex is the principal food store in the underground part of the perennial ryegrass plant, the available food is removed from there and transferred to the growing points. As a result, the cortical epidermal layer is left without its supporting tissues and eventually sloughs off.

The sloughing commences first at the proximal region of the root excluding the portion of about one inch immediately adjoining the base of the plant where secondary root-hair development may take place on the persistent cortical epidermis (Jacques 1939).

The sloughed area extends downwards during November and through the summer. Weinmann (1952) has shown that food reserves in the storage organs may increase or decrease in proportion to the demands made on them by the aerial parts. Reserves are utilized not only in spring, but whenever new aerial growth is produced after defoliation. Effects of repeated defoliations are cumulative, and progressive depletion of reserves will bring about a reduction in the weight of storage organs.

From observations on perennial ryegrass in New Zealand, it would appear that when drastic withdrawals of carbohydrate reserves from the

cortex of the roots takes place (October), the withdrawals are so complete that the cortical epidermis collapses and ultimately sloughs off, leaving that portion of the root incapable of acting any longer either for absorption or storage. As this region extends as a result of summer demands on any reserves that may remain, only the distal portion of the root, which is situated in the moister subsoil, is able to function in procuring water and solutes.

With the onset of autumn rains (March–April) a new vigorously absorbing root system develops from the crown and the need for the deeper roots passes. Furthermore, with a rise in the water table in late winter together with a decrease in the amount of oxygen available to the roots, these deeper roots find difficulty in remaining alive, and mortality among them is high.

The Changing Function of the Roots

When new roots are initiated from the crown between March and October, they are extremely active absorbers of plant-food materials. They are highly organized for absorption in a region where normally there is an ample water and food supply. They are well covered with root hairs and they are capable of storing carbohydrate food reserves at an early stage in their development. Klapp (1937) showed that during the growth of young plants of *Dactylis glomerata* and *Poa pratensis* the quantity of carbohydrate in the root and storage organs increased to a greater extent than did the quantity of total dry matter.

As the roots penetrate deeper, they become more branched and enter a region less rich in plant-food materials, but with a more reliable summer water supply. It would seem therefore that, in the young stage, roots are organized to supply mineral foods and water to the plant and to act as storage organs. As the root extends downwards, the deeper layers are reached coincident with the possibility or occurrence of drought periods affecting the upper layers of the soil. They are thus in a region where water is ample but where mineral supplies are generally scarce. As the demands of rapid spring and early summer growth have been met (in part) by utilizing the reserves of food stored during the periods of slower growth, the main demand is for sufficient water to prevent desiccation during the dry months of the year, when the plant is considerably less active in its growth. Even assuming there was sufficient moisture in the upper layers of the soil to enable absorption to take place, these roots could not avail themselves of it, for the absorptive capacity has been lost with the sloughing of the cortex. The roots that exist in the deeper layers during summer therefore are mainly organized for water supply. This function ceases to be important with the autumn rains when a new set of roots forms in a more fertile region with an adequate water supply. It is possible that very few of the deeper roots of perennial ryegrass, which supplied the plants with water in summer, continue to live through the winter; however, roots formed late in the March-October period, which did not pass through the complete cycle from mineral suppliers to water suppliers, might continue to function in spring and become water suppliers during the next summer. The root system of ryegrass therefore follows the annual cycle of renewal as stated by Stuckey.

FACTORS INFLUENCING LONGEVITY

From the time of germination in March, roots continue to descend into the moister and deeper soil layers. Unless the water table is very high in winter and early spring, it should not normally interfere with the downward growth of new roots produced from March onwards in the first season of growth. By summer, some of the roots may have penetrated down to 6 ft. or even more. With a rise in the water table from May these deeper roots are in danger of being killed by excess of water while the upper portion of the root has deteriorated into a channel along which the solutes pass, and is no longer capable of responding to the stimulus of the removal of the terminal growing point. The roots that have already penetrated deeply are on the whole not in a state to regenerate near the surface and have mostly lost their cortical tissue. "When a root has reached the stage when the cortex sloughs from around the stele there is little, if any, response to root severance" (Jacques 1944). This statement would apply also to those roots that lose their main and lateral terminal growing points through excess of water. Such roots as are incapable of responding to the stimulus of the removal of the terminal growing point(s) consequently die off.

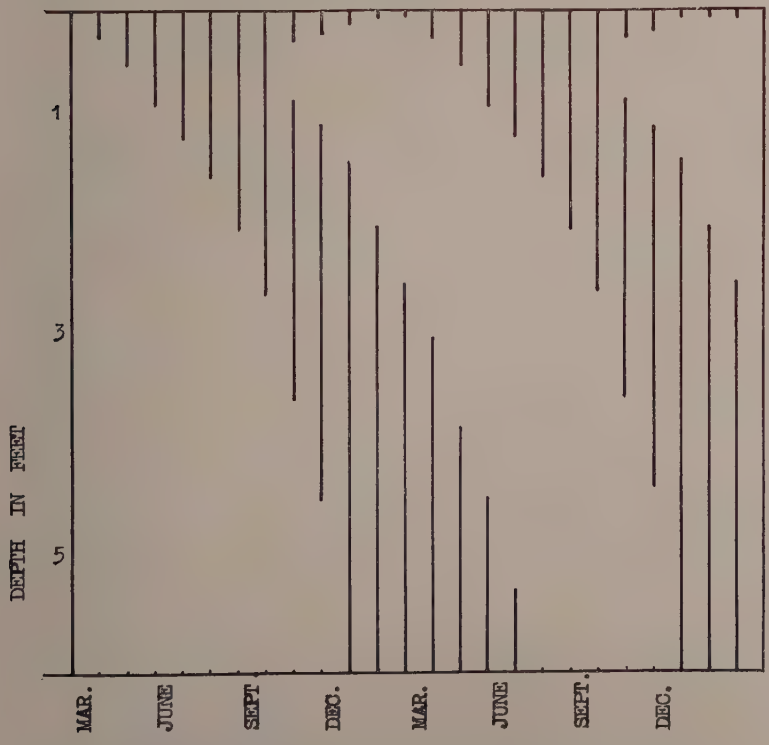

Fig. 1.—Idealized diagram indicating regions of absorption of perennial ryegrass roots. The lines show the region where absorption is taking place, the blanks represent where cortex deterioration or where death through rising water table or redundance have taken place.

Furthermore, if there is a barrier to their penetration into the deeper soil, the roots are likely to be killed during the dry period in summer. Likewise, if a soil becomes too dry, root growth will not penetrate into it (Jacques and Edmond 1952).

Weaver and Zink (1946) have shown that the rate of defoliation may influence the longevity of roots and, as pointed out by Jacques (1937), the amount of root following repeated defoliations is adjusted to the level of the leafage that is allowed to remain on the plant.

Figure 1 is an idealized version of the root behaviour in perennial ryegrass when grown on a deep silt loam at Palmerston North. It shows the development of the root system of plants from seed sown in March and traces the root penetration and deterioration over a 2-year period.

From March to December, and possibly to February, there is a continued downward expansion of the root system as shown by the dark lines. Sloughing of the cortex commences about October and continues from near ground level downwards through the drier months of spring and summer. The sloughed areas are depicted as breaks in the dark lines. The same process is repeated annually. Some mortality would occur from the May–June period (with a possible maximum in August) from a rise in the water table which would meet the downward-moving cortical deterioration.

Discussion

Stuckey (1941) divided the grass species she studied into those with "annual" and those with "perennial" roots. Perennial ryegrass came into the "annual" category.

In studies on grass roots at this centre, it has been apparent that while an element of variability exists in root behaviour, primarily in response to environmental changes, there is a clear pattern of root formation and replacement which is annual in character.

The text of this article gives an idealized version of the root behaviour in perennial ryegrass, and simplifies the root pattern to the extent that the whole of the root system could be replaced in a little over 12 months.

It is clear however (Fig. 1) that the deeper-seated roots are functioning for some time after the new clonal roots appear in March, and that their fate is decided either by a rising water table during winter or by the fact that sufficient food and water to satisfy the plant's needs can be obtained by the new white roots, so that the deeper-seated roots become redundant. Furthermore, they are very essential in a dry autumn when the moisture content of the surface soil is insufficient for the growth of new roots. The pattern then is an annual one with some overlapping of the deeper-seated roots into the succeeding period. As shown by Jacques and Schwass (1956), the estimated mortality of perennial ryegrass roots in a wetter-than-normal year was 68%.

It is suggested that as roots grow older and deeper they alter their function from "feeding" roots (water and minerals) to essentially "water" roots. This is based on the fact that the cortex deteriorates to such a degree over most of the roots' length that most of the absorbing surface left is a limited region at the distal end and that part which is

situated in the subsoil where (mainly) available minerals are more scarce than in the surface layers of the soil.

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INCORPORATION OF FUNGICIDES IN FRUIT-SETTING SPRAYS FOR CONTROL OF *Botrytis* FRUIT ROT IN GLASSHOUSE TOMATOES

I. INTRODUCTION AND SCREENING TRIALS

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(Received for publication, 15 November 1955)

Summary

Under conditions prevailing in New Zealand, *Botrytis cinerea* Pers. frequently becomes established on flower petals of glasshouse tomatoes sprayed with fruit-setting growth substances. Infection then enters the fruits causing severe losses. Heavy sporulation on rotted fruits encourages early and rapid build-up of infection which may lead to epidemic conditions in a crop. A method was devised to overcome this type of fruit infection by protecting petal surfaces with fungicides added to the growth-substance sprays.

A laboratory method was developed to assess the ability of *B. cinerea* to establish on and penetrate dead petals after spraying with mixtures of growth substance and fungicides. Materials effective in the laboratory tests were further screened in tests with pot-grown tomato plants.

Materials based on ferbam, thiram, and ziram, at two to three times the normally recommended strengths gave almost complete control in both sets of trials.

INTRODUCTION

Until recently, crop losses caused by *Botrytis cinerea* Pers. in commercial glasshouse tomatoes in the Auckland province were slight. What losses there were tended to occur towards the end of the season. Over the last six years, however, outbreaks of *Botrytis* have commonly been on an epidemic scale. Attempts to control outbreaks by use of copper, sulphur, or salicylanilide sprays have proved unsuccessful.

B. cinerea, a wound parasite, must first develop on damaged, ageing, or leaf-mould-infected* tissue which is not usually present in quantity until relatively late in the life of a crop. Dead petals provide suitable infection sites, but with pollinated flowers they are normally shed rapidly from the style ends of developing fruits. Where *Botrytis* has become well established in a glasshouse, dead petals may become infected before they are shed, leading to fruit rot or blemish (Figs. 1, 2). Infected petals may fall and lodge on leaves and maturing lower-truss fruit (Fig. 3). In glasshouses where *Botrytis* attacks have reached an epidemic scale in recent years, there has been a history of a considerable amount of lower-truss fruit rot earlier in the season. Most of this early fruit infection on basal trusses follows use of fruit-setting sprays which cause petals to be retained under the calyx or on the side of the fruit. When these petals die they provide ideal entry points for *B. cinerea* (Figs. 4, 5). The resulting infection causes heavy fruit losses from the early, artificially set trusses, and since spore production is prolific on tomato fruits, it also has the effect of increasing the rate of build-up of *Botrytis* in a crop.

* Leaf mould = *Cladosporium fulvum* Cke.

Fig. 1.—Style-end rot on young fruit established through dead petals before they are shed.

—*Photo: J. W. Endt.*

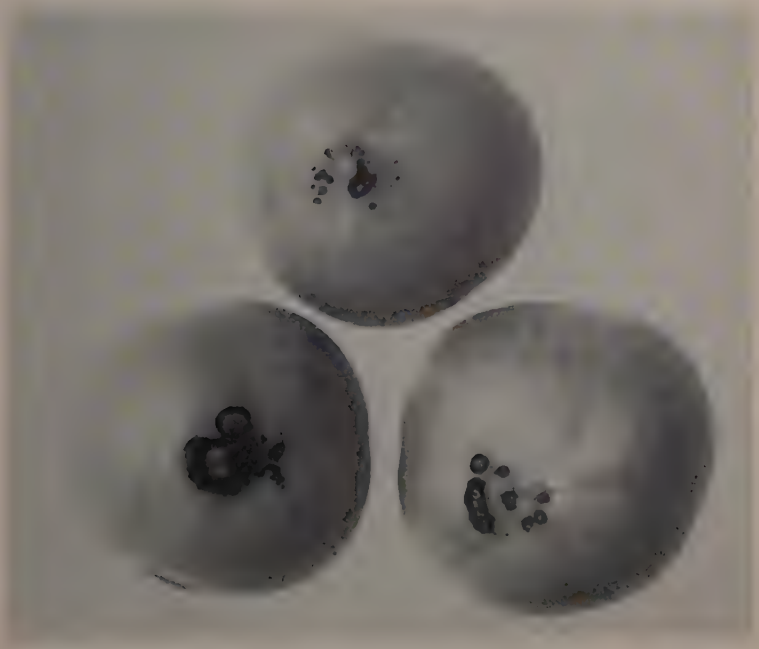

Fig. 2.—Small blemishes on style-end of young fruit caused by *Botrytis cinerea* growing on dead petals before they were shed.

—*Photo: S. R. Rumsey.*

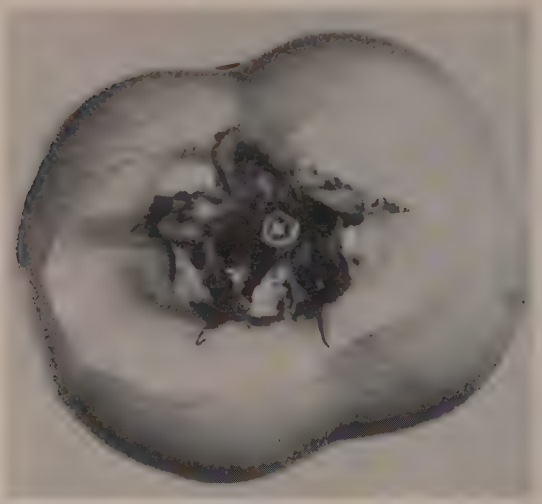

Fig. 3.—Fruit rot caused by *Botrytis cinerea* entering through dead petals retained below calyx.
—*Photo: J. W. Endt.*

Fig. 4.—*Botrytis*-infected petals on side of tomato set with growth substance.
—*Photo: J. W. Endt.*

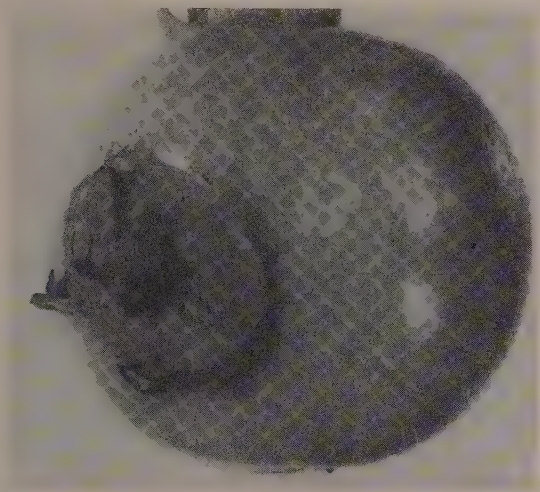

Fig. 5.—*Botrytis* rot on tomato caused by lodging of infected petals from upper-truss flowers. ("Ghost spot" lesions indicate intensity of air-borne inoculum present in the house.)

—*Photo: J. W. Endt.*

One method of control of the fruit-rot phase of the disease is hand removal of dead petals, a tedious and scarcely practicable procedure. Sometimes petals adhere so firmly under the calyx that removal is difficult and fruit is liable to be damaged.

It was considered that a more suitable means of control would be use of a fungicide, compatible with the growth-substance sprays, which would protect petals against *B. cinerea* infection without damaging the flower. A preliminary account of an investigation of this aspect of control has already been published (Newhook and Davison, 1953). The following series of papers deals with therapeutant screening trials, compatibility trials, and field-scale tests of the combined sprays.

MATERIALS

Materials tested are listed below, with the proprietary forms used indicated in brackets.

β-naphthoxyacetic acid (Fulset)
Bordeaux mixture
Copper oxychloride (Cuprox, 50% a.i.)
Cuprous oxide (Perenox, 50% a.i.)
Ferbam—ferric dimethyldithiocarbamate (Fermspray 70% a.i.)
Ziram—zinc dimethyldithiocarbamate (Zirospray, 70% a.i.)
Thiram—tetramethyl thiuram disulphide (Thirospray, 50% a.i.)
Nabam—disodium ethylene bisdithiocarbamate (Dithane 14, 19% a.i.)
Zineb—zinc ethylene bisdithiocarbamate (Dithane 78, 65% a.i.)
Dichlone—2, 3-dichloro-1, 4-napthoquinone (Phygon XL, 50% a.i.)
Chloranil—tetrachloro para benzoquinone (Tetroc)
Captan—n-trichloro methylthio tetrahydrophthalimide (Flit 406, 50% a.i.)

Phenyl mercury chloride (Ascospray and Venturicide, both 2% a.i.)

Salicylanilide (Shirlan AG, 25% a.i.)

8-quinolinol

8-quinolinol sulphate

8-quinolinol benzoate

Copper derivative of 8-quinolinol

Zinc derivative of 8-quinolinol

Maleic hydrazide

8-azaadenine

8-azaguanine

8-azahypoxanthine

Thiouracil

Experimental

Laboratory Screening Trials

To provide a convenient test surface on which factors governing spray retention and infection would be more closely related to those in the field, the following method was devised:

Stainless-steel holders were constructed (Figs. 6 and 7) with either one or ten holes $\frac{1}{16}$ in. diameter. Petals from fully expanded tomato flowers were attached across the undersides of the holes with cellulose acetate glue, care being taken not to clog holes or coat petal surfaces in that region. Holders with petals were sterilized for half an hour in petri dishes at an oven temperature of 110°C. When cool, holders were taken from the dishes and the surfaces with the petals sprayed with mixtures of fungicide and growth substance. Excess spray was blotted from the edges, and petal holders were allowed to dry out in the dishes. The exposed surfaces of the petals were then dusted with dry *B. cinerea* spores. After dusting, holders were removed to a sterile petri dish, the bottom of which was lined with damp filter paper. A small block of plain agar was then placed over each hole (Fig. 6) and dishes incubated at 21°C.

Within two days, mycelial growth appeared on petals treated with ineffective materials. Three to seven days later *Botrytis* growth was evident on the agar blocks. Where necessary, sections were cut through the blocks to check that growth came from the petals and not from contamination.

Dichlone 0.05% or 0.075% was usually used as a standard effective material while checks consisted of petals sprayed with growth substance alone. The aim was to test each material more than once, increasing concentrations to an effective level where necessary, except when a material was discarded because of phytotoxicity or incompatibility in tests on plants run concurrently with the laboratory screening trials.

Results of a series of typical tests are presented in Table 1.

TABLE 1. GERMINATION AND PENETRATION BY *B. cinerea* ON DRIED TOMATO PETALS SPRAYED WITH GROWTH SUBSTANCE PLUS FUNGICIDES

Trial	Treatment	No. of Petals with: Complete Control	Growth on Petal Only	Penetration to Agar Block
I.	Fulset check	0	0	8
	,, + dichlone 0.05%	8	0	0
	,, + dichlone 0.025%	5	0	3
	,, + dichlone 0.01%	0	0	8
	,, + ferbam 0.07%	4	0	4
	,, + thiram 0.2%	0	0	8
	,, + thiram 0.1%	0	0	8
	,, + 8-quinolinol (saturated soln.)	0	0	8
	,, + 8-quinolinol benzoate (sat. soln.)	0	8	0
	,, + 8-quinolinol sulphate 0.4%	0	8	0
	,, + 8-quinolinol sulphate 0.2%	0	1	7
II.	Fulset check	0	0	8
	,, + chloranil 0.1%	0	0	8
	,, + cuprous oxide 0.25%	0	0	8
	,, + dichlone 0.05%	8	0	0
	,, + ferbam 0.35%	8	0	0
	,, + nabam 0.076%	7	0	1*
	,, + salicylanilide 0.75%	0	0	8
	,, + thiram 0.3%	6	0	2
	,, + ziram 0.14%	4	2	2
	,, + 8-quinolinol (sat. sol.)	0	1	7
	,, + 8-quinolinol benzoate (sat. sol.)	0	0	8*
	,, + 8-quinolinol sulphate 0.4%	8	0	0
III.	Fulset check	0	0	8
	,, + copper 8-quinolinol (sat. sol.)	0	0	8
	,, + dichlone 0.05%	8	0	0
	,, + maleic hydrazide 0.15%	0	0	8
	,, + phenyl mercury chloride 0.002%	8	0	0
	,, + zineb 0.065%	0	0	8
IV.	Fulset check	0	0	9
	,, + copper 8-quinolinol (suspension) 0.025%	0	5	4
	,, + copper 8-quinolinol (suspension) 0.1%	5	4*	0
	,, + 8-quinolinol benzoate (suspension) 0.1%	7	2*	0
	,, + phenyl mercury chloride 0.002%	6	2	1
	,, + phenyl mercury chloride 0.004%	9	0	0
	,, + thiouracil 0.01M	0	0	9
	,, + 8-azaadenine 0.01M	9	0	0
	,, + 8-azaguanine 0.01M	0	0	9
	,, + 8-azahypoxanthine 0.01M	0	0	9
V.	Fulset check	0	0	8
	,, + dichlone 0.075%	8	0	0
	,, + 8-azaadenine 0.003M	5	0	3*
	,, + 8-azaadenine 0.001M	0	2*	6
	,, + 8-azaadenine 0.0003M	0	0	8
	,, + 8-azaadenine 0.0001M	0	0	8
	,, + 8-azaadenine 0.00003M	0	0	8
	,, + 8-azahypoxanthine 0.003M	0	0	8
	,, + 8-azahypoxanthine 0.003M + 8-azaadenine 0.003M	0	5	3
	,, + 8-azahypoxanthine 0.01M + 8-azaadenine 0.01M	8	0	0
	,, + 8-azaadenine 0.01M	8	0	0

* Slight growth only.

Fig. 6.—Stainless-steel holder, top view, showing agar block placed above the hole on the other side of which is fixed an inoculated dead petal.

—*Photo: J. W. Endt.*

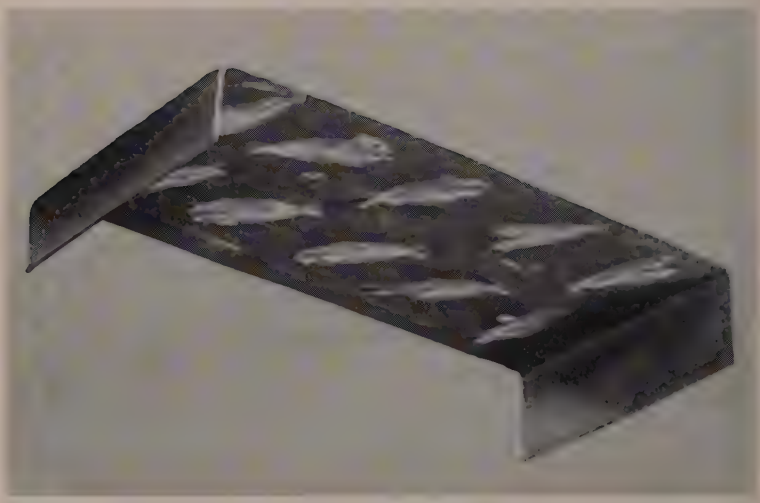

Fig 7.—Stainless-steel holder, bottom view showing several petals glued over holes.

—*Photo: J. W. Endt.*

The following materials gave complete control of *B. cinerea*: captan 0.2%, dichlone 0.05%, ferbam 0.35%, nabam 0.076%, phenyl mercury chloride 0.002%, thiram 0.3%, ziram 0.42%, 0.1% suspensions of copper derivative of 8-quinolinol and 8-quinolinol benzoate (saturated solutions were ineffective), 8-quinolinol sulphate 0.4%, 8-azaadenine 0.01M and a mixture of 8-azaadenine 0.01M and 8-azahypoxanthine 0.01M. To obtain control of *B. cinerea* the concentrations of copper oxychloride and cuprous oxide had to be increased to 1.0–2.5%, 5 to 10 times the strengths necessary for control of other diseases for which these materials are recommended.

Trials on Flowers and Fruits

Only those materials proving effective in the preliminary screening trials were used in these tests. Potentate was the tomato variety used in all trials, grown in 6- or 8-inch pots and stopped just above the second truss.

Fig. 8.—Distribution of ferbam residues on tomato flower which had been sprayed once only, at full bloom.
—*Photo: J. W. Endt.*

Fruit-setting sprays are normally applied only to the upper or inside surface of the flowers. To obtain adequate protection of petals, however, the combined sprays were applied to both upper and lower surfaces. In the earliest trials of this series it was noticed that sprays applied to the lower surfaces of fully open flowers did not reach the bases of the petals beneath the calyx. When petals wilted some days after setting, unprotected areas became obvious when such materials as Fermspray were applied since they left a dark residue (Figs. 8, 9). Adding detergents did not cause sprays to penetrate further beneath the calyx. In subsequent tests the technique was therefore modified as follows:

Fig. 9.—Tomato fruit developing from flower set with a single application of combined spray, showing incomplete distribution of ferbam residues.

—*Photo: J. W. Endt.*

Trusses were sprayed with combinations of fungicide and growth substance when several flowers were open and again 7 to 10 days later when bases of the petals were exposed. Check trusses were treated with growth substance only. When the largest fruits on the trusses were about half grown, all fruits were picked, arranged in replicated groups each containing a full range of fruit sizes, and placed in a tray, dead petals uppermost. Flowers that had opened later and had thus been sprayed once only were not used in these tests. The fruit was sprayed with a heavy spore suspension of *B. cinerea,* placed in a humidity chamber for six days and then removed to a glasshouse bench. Records of petal and fruit infection were taken at intervals.

Two examples of trials based on the above method are presented.

Trial 1. Treatments and results are listed in Table 2. Sprays were applied twice ("double spray") in combination with growth substance, except for one treatment which consisted of a single application of growth substance plus dichlone, 0.05%.

TABLE 2. INFECTION ON TOMATO FRUITS INOCULATED WITH *B. cinerea* AFTER SETTING WITH GROWTH SUBSTANCE PLUS FUNGICIDE

Treatment	No. of Days after Inoculation					
	6*		13†		17†	
	Angle	%	Angle	%	Angle	%
Fulset check	49.6	58.0	50.4	60.0	84.7	96.0
„ + dichlone 0.05% single spray	19.0	13.3	23.1	20.0	51.3	55.6
„ + dichlone 0.05% double spray	3.5	1.8	24.5	18.2	45.6	50.9
„ + ferbam 0.35% double spray	0	0	0	0	0	0
„ + thiram 0.3% double spray	0	0	0	0	3.9	2.2
„ + zineb 0.065% double spray	61.7	76.2	58.8	63.1	78.6	90.5
„ + ziram 0.28% double spray	0	0	11.4	6.7	27.7	21.7
„ + 8-quinolinol benzoate sat. double spray	41.1	43.3	32.4	30.0	55.0	66.7
„ + 8-quinolinol sulphate 0.4% double spray	44.3	48.9	31.9	33.3	65.0	73.3
Sig. diff. 5%	12.3		17.2		17.8	
1%	16.8		23.1		24.0	

* Only petal infection showing at this stage.

† Only fruit-rot data presented.

Column 2 records percentage infections on petals upon removal from the humidity cabinet. Column 4 records percentage fruit rot on the thirteenth day, after which unrotted fruits were replaced in the humidity cabinet for a further four days. Column 6 records percentage fruit rot on completion of the experiment.

Ferbam 0.35% and thiram 0.3% were outstanding amongst materials tested. Ziram 0.28% and dichlone 0.05%, although giving good control during early stages of the experiment gradually lost effectiveness while the 8-quinolinol compounds gave slight control throughout. Zineb 0.065% was ineffective and in addition caused damage to petals and young fruits.

Data recorded from the second trusses were similar to those just described except that 8-quinolinol sulphate 0.4% gave complete control. On both trusses, however, this material produced marked scorching of petals and anthers.

Trial 2. In this trial, double applications of combined sprays were made to upper and lower aspects of flowers.

Results are presented in Table 3.

Thiram 0.3%, ferbam 0.35%, and ziram 0.28% gave outstanding control of *B. cinerea,* comparable with that obtained by hand removal of dead petals. Though they reduced infections significantly, the 8-quinolinol compounds 0.2% and dichlone 0.075%, were not as effective as the above three treatments. Other materials were ineffective. Except

TABLE 3. Incidence of Fruit Rot on Tomatoes Set with Growth Substance plus Fungicide

Treatment	Friut Rot after 18 Days	
	Angle	%
Fulset check	65.9	82.8
,, + petals removed by hand	4.1	1.9
,, + dichlone 0.075%	33.7	32.5
,, + ferbam 0.35%	3.8	1.8
,, + phenyl mercury chloride 0.003%	61.0	75.0
,, + thiram 0.3%	0	0
,, + ziram 0.28%	7.7	3.6
,, + copper 8-quinolinol 0.2% (susp.)	25.2	18.8
,, + 8-quinolinol benzoate 0.2% (susp.)	24.9	18.8
,, + 8-azaadenine 0.003M	59.7	73.5
,, + 8-azaadenine 0.003M + azahyphonanthine 0.003M	62.2	78.0
Sig. diff. 5%	13.7	
1%	18.7	

for the quinolinol compounds and dichlone, which respectively caused severe and slight scorching of petals and anthers, and for phenyl mercury chloride, which appeared to inhibit truss development, no materials induced plant injury.

Conclusions

The technique developed for the laboratory screening trials provided a practicable method of testing the effectiveness of fungicidal residues on host tissue. Trials using this technique showed that, when sprayed with fruit-setting growth substances on surfaces of dried petals, several fungicides will prevent establishment of *B. cinerea*. The most promising appeared to be some of the organic fungicides, which proved superior to materials in general use as overall sprays on tomatoes under glass.

Of materials shown to be effective in the screening trials, ferbam 0.35%, thiram 0.3%, and ziram 0.28% were most promising when applied with growth substance to flowers. Two applications at an interval of 7 to 10 days were found to be necessary to obtain adequate petal protection. None of the systemic fungicides tested proved satisfactory.

Acknowledgement

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Reference

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INCORPORATION OF FUNGICIDES IN FRUIT-SETTING SPRAYS FOR CONTROL OF *Botrytis* FRUIT ROT IN GLASSHOUSE TOMATOES

II. COMPATIBILITY OF MIXTURES

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Summary

Tests to ascertain the compatibility of various fungicides with fruit-setting chemicals have been carried out on pot-grown tomatoes.

Addition of ferbam 0.28–0.42%, thiram 0.3%, ziram 0.3%, captan 0.2%, and dichlone 0.05% to β-napththoxyacetic acid sprays had no adverse effect on size, number, yield, or quality of fruit. Similar results were obtained in a single trial in which the first four were added to sprays containing parachlorophenoxyacetic acid.

Copper derivative of 8-quinolinol 0.1% and 0.2% seriously reduced fruit size, yield, and quality. Ferbam 0.56% and dichlone 0.1% when added to β-napththoxyacetic acid reduced yields and fruit size in the first truss of one experiment.

Introduction

The effectiveness of certain fungicides when added to a fruit setting spray, in reducing fruit rots caused by *Botrytis cinerea* Pers. in glasshouse tomatoes has been demonstrated (Newhook and Davison 1956a). It was necessary to determine whether these fungicides reduced effects of the growth substance or caused injury to trusses or fruit. Results of experiments over three seasons to ascertain the compatibility of various fungicides with fruit-setting sprays are given below.

Methods

Potentate tomato plants, grown in 10-in. pots in a glasshouse, were used in all experiments. Each treatment was carried out on 7 to 9 plants. Plants were stopped after development of the second or third truss and flowers emasculated before natural set occurred. Fruit-setting sprays with or without added fungicides were applied within twenty-one days of emasculation of the first-opening flower on each truss. Formulation of the fungicides used has been listed previously (Newhook and Davison 1956a). Two commercial fruit-setting preparations were tested, one based on β-napthoxyacetic acid (Fulset) and the other on parachlorophenoxyacetic acid (Tomatone).

In the 1952, trial, upper and lower surfaces of flowers were thoroughly wetted with a single application of the combined sprays.

In most treatments of the 1953 and 1954 trials an additional spray of fungicide was applied alone at the same concentration 7 to 10 days after the combined sprays, since further work had shown that this "follow up" spray was essential to obtain optimum control of *B. cinerea*. In two treatments in the 1953 trial, trusses were set with growth substance alone and sprayed 7 to 10 days later with ferbam at high concentrations (0.56% and 0.84%).

TABLE 1. YIELDS FROM TOMATO PLANTS TREATED WITH TRUSS SPRAYS OF FRUIT-SETTING CHEMICALS APPLIED ALONE OR WITH FUNGICIDES

Treatment	First Truss			Whole Plant*		
	Yield per Plant (oz.)	Mean Fruit Weight (oz.)	Mean No. Fruit per Plant	Yield per Plant (oz.)	Mean Fruit Weight (oz.)	Mean No. Fruit per Plant
1952 Trial						
Fulset (20:1) Check	13.1	1.58	8.2	30.3	1.83	16.3
" + copper 8-quinolinol 0.1%	7.3	0.94	8.3	15.7	1.05	15.9
" + copper 8-quinolinol 0.2%	7.6	0.91	8.7	17.1	1.07	16.2
" + dichlone 0.075%	11.5	1.43	7.9	26.7	1.89	14.2
" + dichlone 0.1%	8.8	1.11	7.7	27.8	1.72	15.9
" + ferbam 0.35%	11.7	1.84	6.7	28.9	2.02	14.9
" + ferbam 0.56%	8.3	1.12	9.3	27.9	1.77	18.1
" + thiram 0.3%	13.0	1.72	7.8	30.7	1.92	16.2
" + ziram 0.28%	15.9	1.92	8.7	35.0	2.27	16.0
Sig Diff. 5%	4.2	0.42	2.9	7.4	0.48	4.2
1%	5.6	0.56	3.9	9.8	0.63	5.6
1953 Trial						
Fulset (20:1) Check	10.5	1.72	6.4	25.1	1.69	15.4
" + captan 0.2%	13.9	1.69	8.3	39.2	1.95	20.9
" + dichlone 0.1% plus spray with	9.5	1.63	5.9	30.5	1.82	17.0
" + ferbam 0.35% fungicide at	12.3	1.59	7.8	29.0	1.81	16.5
" + ferbam 0.46% same	10.2	1.81	5.7	33.4	1.87	18.4
" + ferbam 0.56% concentration	10.7	1.66	6.6	29.6	1.84	16.1
" + thiram 0.4% 7-10 days later	14.2	1.70	8.6	37.5	1.88	20.0
" alone + ferbam 0.56% 7-10 days later	13.3	1.69	7.7	27.0	1.71	15.7
" alone + ferbam 0.84% 7-10 days later	14.2	1.63	8.7	34.1	1.81	19.2
Sig Diff. 5%	3.8	0.37	2.2	9.5	0.34	5.3
1%	5.1	0.49	2.9	12.6	0.45	7.1
1954 Trial						
Fulset (20:1) Check	20.4	2.31	9.0	65.9	2.37	28.4
" + captan 0.2% plus spray with	17.3	2.49	7.3	68.9	2.72	25.6
" + ferbam 0.28% fungicide at	19.4	2.24	8.7	68.9	2.36	29.0
" + ferbam 0.42% same	19.9	2.22	9.5	71.7	2.37	31.7
" + thiram 0.3% concentration	19.3	2.17	8.8	60.7	2.20	27.6
" + ziram 0.42% 7-10 days later	16.6	2.21	7.5	64.4	2.46	26.4
Tomatone (150:1) Check	19.6	2.27	8.9	62.6	2.25	28.1
" + captan 0.2% plus spray with	19.6	2.33	8.5	73.4	2.25	33.0
" + ferbam 0.28% fungicide at same	16.8	2.26	7.9	63.8	2.09	31.0
" + thiram 0.3% concentration	20.3	2.17	9.9	70.2	2.42	29.2
" + ziram 0.42% 7-10 days later	15.4	1.91	8.3	52.6	1.97	27.1
Sig Diff. 5%	6.1	0.49	3.1	24.1	0.40	5.7
1%	8.1	0.64	4.1	32.0	0.53	7.6

* Two trusses in 1952 and 1953, three in 1954.

Checks in all trials were sprayed with growth substance alone.

Weights and counts of fruit harvested were recorded, and from these a mean fruit weight was calculated. Observations were also made on such aspects of fruit quality as hollowness, distortion, texture, etc.

Details of treatments and results are given in Table 1.

RESULTS

No treatment reduced the number of fruits set.

Both concentrations of copper derivative of 8-quinolinol, 0.1% and 0.2%, reduced total yields and fruit size when mixed with growth substance (β-naphthoxyacetic acid). Fruits were small, dull skinned, lacking in pulp, resembling those that sometimes form on emasculated unsprayed flowers.

Dichlone 0.1% and ferbam 0.56% reduced yield and fruit size in the 1952 trial but the effect was confined to the first trusses of plants so treated. Losses appeared to be largely compensated for by an increase in the weight of the second-truss fruits so that total yield and mean weight for the two trusses were not significantly less than those of the checks. Fruit quality was not adversely affected nor did similar treatments produce any adverse effect in subsequent seasons. There was no suggestion of incompatibility with the lower strengths of either dichlone or ferbam.

None of the sprays injured trusses externally.

CONCLUSIONS

While some fungicides have been shown to cause damage when applied with growth substances (Newhook and Davison 1956a, b), trials in three seasons indicated that captan 0.2%, dichlone 0.075%, ferbam 0.28–0.46%, thiram 0.3–0.4%, and ziram 0.28–0.46% can be mixed safely with β-naphthoxyacetic acid. This conclusion is suggested by observations in other trials and commercial crops to which combined sprays have been applied during four seasons. Results obtained from one trial indicated that captan 0.2%, ferbam 0.28%, thiram 0.3%, and ziram 0.42% may be added safely also to parachlorophenoxyacetic acid.

ACKNOWLEDGEMENT

The authors wish to thank Dr. H. R. Thompson of the Applied Mathematics Laboratory, Department of Scientific and Industrial Research, for statistical analyses.

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INCORPORATION OF FUNGICIDES IN FRUIT-SETTING SPRAYS FOR CONTROL OF *Botrytis* FRUIT ROT IN GLASSHOUSE TOMATOES

III. TESTS IN COMMERCIAL HOUSES

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Summary

Experiments under commercial growing conditions were carried out on winter and spring tomato crops to control the fruit rot that follows establishment of *Botrytis cinerea* Pers. on dead petals.

It was shown that to secure maximum protection truss sprays should be applied in such a way that each flower receives at least two applications of fungicide, the first in a combination spray of growth substance and fungicide, the second in a spray of fungicide alone 7 to 12 days after setting. The results showed that the latter can be supplied either by special truss sprays or by overall sprays for *Botrytis* control. Overall sprays did not satisfactorily replace inclusion of fungicides in growth substance sprays.

Ferbam 0.35% and thiram 0.3% gave best control in combined sprays.

Introduction

Preliminary trials (Newhook and Davison, 1956: Davison and Newhook, 1956) have shown that certain fungicides, when added to fruit-setting agents, reduce fruit infection with *Botrytis cinerea* Pers. without adversely affecting fruit size, number, yield, or quality. Indications were obtained that these sprays should be applied at specific times in relation to flower and fruit development to protect completely both surfaces of petals retained at the calyx end of developing fruit.

To test effects of these sprays under commercial conditions, trials were carried out in 1952 and 1953. Preliminary results have been published (Newhook and Davison, 1953). A detailed account of the tests is given below.

Experimental

Potentate tomato plants were set out in unheated glasshouses as winter or early-spring crops in Auckland. There were five or six replicates of single- or double-row plots for each treatment. Plots contained 12, 15, or 25 plants.

Formulation of the fungicides used has been listed previously (Newhook and Davison, 1956).

Application of Sprays

Hand atomizers were used to apply combined sprays or sprays of fungicides alone to the trusses. Trusses in check plots were sprayed with growth substance alone. In the first trial the method of treatment was the same as that described earlier (Newhook and Davison, 1956). A single combined spray was applied to each truss when 75% of the flowers were fully open. This spray was followed by an application of

fungicide alone 7 to 12 days later. In the second trial, combined sprays were applied at two stages of truss development; the first application thoroughly wetted all parts of the trusses, and the second only the upper surfaces of flowers; an application of fungicide alone was made 7 to 12 days after the second combined spray. In the third trial, the method of application was varied with a view to determining the minimum practical truss-spraying procedure required to give adequate control of *B. cinerea*.

Infection

Heavy infection developed in one (Avondale) trial of a series laid down in 1952, all depending entirely on natural infection. Trial plots were randomized on each side of a glasshouse and were separated by buffer plots consisting of double rows in which the trusses were set with growth substance alone. In two trials laid down in 1953, one at Mt. Albert and one at Oratia, plants were subjected to uniform infection by spraying with *B. cinerea* spore suspensions and by distributing sporing cultures of *B. cinerea* at regular intervals along rows.

Infected fruits were removed and recorded before lateral spread from contact occurred in the trusses. The data thus refer almost entirely to infection establishing directly through retained dead petals.

Weights of fruit harvested from treated trusses were recorded and observations made on fruit quality.

RESULTS AND DISCUSSION

Details of treatments and results of the three trials are given in Table 1.

Results confirm those obtained in preliminary tests (Newhook and Davison, 1956). They indicate that fungicidal treatments applied to trusses at the time of fruit setting markedly reduce fruit losses caused by *B. cinerea* in commercial crops. A high degree of control was maintained on treated trusses despite inoculation or the presence of heavy natural infection on adjacent buffer rows. Reductions of up to 98% in the number of rotted fruits were secured with some treatments.

Of the materials tested, ferbam 0.35% gave best control in all trials. Dichlone at Avondale and captan at Mt. Albert, although as effective as ferbam in the early stages of the trials, appeared to lose efficacy later. A reduction of 82% infection with the ferbam treatment in the Mt. Albert trial compared favourably with 90% control secured by the laborious method of hand removal of petals. Losses in the latter treatment were the result of infection establishing through portions of petals retained too tightly under the calyx for complete removal and also some sepal tip infection.

Three conclusions may be drawn concerning the practical application of combined fungicide–growth substance sprays, as shown by the Oratia trial.

1. Adoption of an overall spray programme alone does not give as good control of fruit rots as the complete truss-spray programme alone.

TABLE 1. INCIDENCE OF FRUIT ROT AND YIELDS OF TOMATOES SET WITH GROWTH SUBSTANCE PLUS FUNGICIDE

Treatments	Fruit Rot: Per 25 Plants	Fruit Rot: Control %	Mean Yield (lb.): Per Plant	Mean Yield (lb.): % Increase Over Check
Avondale 1952 (three trusses)				
1. Fulset check	162.1	—	2.60	—
2. ,, + dichlone 0.075% + follow-up spray	76.9	52.6	3.13	20.1
3. ,, + dichlone 0.1% + follow-up spray	87.0	46.3	3.10	19.0
4. ,, + ferbam 0.35% + follow-up spray	36.7	77.4	3.30	26.9
Sig. diff. 5%	29.3		0.26	
1%	40.5		0.36	
	Per 12 Plants			
Mt. Albert 1953 (five trusses)†	No. / Log.			
1. Fulset check	125 / 2.09	—	2.03	—
2. ,, Petals removed by hand	12 / 1.09	90.4	2.38	17.3
3. ,, + captan 0.15% + follow-up spray	57 / 1.76	54.4	2.14	5.4
4. ,, + dichlone 0.075% + follow-up spray	40 / 1.60	68.0	2.57	26.6
5. ,, + ferbam 0.35% + follow-up spray	22 / 1.35	82.4	2.44	20.2
6. ,, + thiram 0.3% + follow-up spray	32 / 1.49	74.4	2.29	12.8
Sig. diff. 5%	0.12		0.32	
1%	0.16		0.44	
	Per 75 Plants			
Oratia 1953 (six trusses)	No. / Log.			
1. Fulset check	191 / 1.55	—	6.65	—
2. ,, + captan 0.15%—complete truss programme	14 / 0.50	92.7	6.98	5.0
3. ,, + thiram 0.3%—complete truss programme	9 / 0.41	95.3	6.70	0.8
4. ,, + ferbam 0.35%—complete truss programme	4 / 0.14	97.9	6.63	—0.3
5. ,, + ferbam 0.35%—fronts of flowers only*	20 / 0.66	89.5	7.10	6.8
6. ,, + overall spray, thiram 0.2%	29 / 0.82	84.8	6.98	5.0
7. ,, + ferbam 0.35% complete truss programme + overall spray, thiram 0.2%	3 / 0.16	98.4	6.92	4.1
8. ,, + ferbam 0.35% fronts of flowers only* + overall spray, thiram 0.2%	6 / 0.28	96.9	7.17	7.8
Sig diff. 5%	0.32		0.40	
1%	0.43		0.54	

† Low yield figures in this trial resulted from heavy infection with leaf mould; no overall sprays or aerosols were applied in trials except where indicated.
* No separate spray to truss with fungicide alone.

2. Use of an overall spray can replace special applications to the lower surfaces of petals in the truss-spray programme, which then involves no more labour than the normal process of fruit setting.

3. If no overall spray is used, a follow-up truss application of fungicide (as used in the complete truss-spray programme) is essential.

Use of combined sprays caused no damage to flowers, save with dichlone, which slightly scorched petals and anthers. The dark residues left on young trusses by ferbam are not a disadvantage. They can be useful in indicating that a truss had been previously sprayed, and soon become insignificant as the fruits expand.

Data from these experiments show that yield increases of up to 27% were obtained through reduction in fruit rots by the use of combined sprays. This was particularly noticeable in the Avondale and Mt. Albert trials, where the incidence of *Botrytis* was much higher than in the Oratia trial. There were no indications that treatments reduced fruit size, thus confirming, in general, the results of the compatibility trials described earlier (Davison and Newhook, 1956).

Leopold and Guernsey (1953) reported that fruit-setting sprays of growth substances applied to back of flowers may increase hollowness of fruits and other abnormalities. No differences in fruit quality between checks and other treatments were observed in the Auckland trials. The possibility of such a loss in fruit quality can be avoided by using the modified truss-spray programme in which sprays containing growth substance are applied only to the upper surfaces of flowers, petal protection being completed by use of overall sprays or truss sprays of fungicide alone applied several days after flowers have been set.

Overall sprays are necessary to control leaf and stem infection but because they cannot be timed in their application to give full coverage to both surfaces of all tomato petals they do not give as good control of fruit rot as the truss spray programme. Both types of spray application are therefore necessary. The reduction in the numbers of infected fruits by the use of the combined truss sprays must, incidentally, increase the efficiency of an overall spray programme by reducing the most important internal source of inoculum. Brook (1956) has indicated suitable treatments for overall sprays.

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A PRELIMINARY REPORT OF GROWTH AND MILK PRODUCTION IN IDENTICAL- AND FRATERNAL-TWIN DAIRY CATTLE

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Summary

Growth data for 28 sets of monozygotic and fraternal twins, together with the milk and butterfat production data for 21 of these sets, are reported.

Analyses of components of variance of these data indicate that maternal environmental factors may be of considerable importance in determining growth but not milk production.

Heritability and repeatability estimates derived in several ways are reported and discussed.

INTRODUCTION

The relationship between genotype and phenotype for milk production has been determined in the past by estimates of repeatability of production for individual animals in herds subject to varying and unknown amounts of environmental variation. Such estimates have also been confounded by the necessity of applying correcting figures to individual two- and three-year-old production records. Heritability estimates for milk production have in the main been based upon dam-daughter and paternal half-sib comparisons, such estimates being made from largely non-contemporary records or from records made in herds subject to varying feeding and management practices. A further disadvantage of these methods of computation is that the total variation is partitioned between genetic and environmental variation only. Thus, the environmental fraction includes, in addition to all true environmental effects, possibly some purely genetic variation derived from dominance and epistasis effects, as well as any variation due to interactions between environment and genotype.

The recent use of identical twin cattle has enabled the total variation in milk production of a herd of cows to be so partitioned that the genetic variance includes that due to non-additive gene effects and to genotype-environment interaction. Using this approach, both Bonnier and Hancock have found repeatability and heritability values of the order of 0.8-0.9[1] whereas the estimates derived in the manner described above have ranged from 0.2-0.4[2]. Naturally, the question arises: What is the cause of the discrepancy between estimates derived by the two methods? This problem has been briefly discussed by Hancock[1] who concluded that it arose mainly from the different degree of environmental control achieved in the two populations from which the statistics were derived. However, four further possible sources of difference exist: the common prenatal environment of the twins, genetic interactions common to the members of a twin set, genotype-environment interactions, and differences in the genetic merit of the herds from which the twins were drawn.

One method whereby the likely importance of the first two of these latter three factors may be assessed is by conducting a uniformity trial where sets of identical twins and sets of fraternal twins are run under uniform conditions within one herd. The results of such a uniformity trial are reported in this paper. The importance of genetic differences between herds and of genotype-environment interactions may be assessed by placing members of identical twin sets in varying environments. The results of this second type of trial will be reported subsequently.

EXPERIMENT

The technique of identical twin collection used at Ruakura involves extensive farmer co-operation, whereby the farmer notifies the Station of the birth of a set of twin calves that are reasonably alike. The farm is visited by a Diagnostic Officer who inspects the twins and, if these appear to be monozygotic[3], they are purchased.

Fifteen sets collected in this manner, together with fifteen sets of fraternal twins were selected such that they had a similar age distribution.

The thirty sets of twins collected were reared by the standard Ruakura technique[4] and calved down at two years of age. They were milked for one complete lactation. As from birth they were subject *entirely* to free grazing conditions, thus the inherited ability of the animal in relation to growth and production and its relation to the seasonal changes in pasture growth were given ample opportunity for expression.

The measurements listed below were made on all animals at weekly intervals from the time they arrived at Ruakura until the end of their first lactation:

(a) Weight (lb.)

(b) Chest girth (cm.)

(c) Belly girth (cm.)

(d) Height over withers (cm.)

(e) Body length (shoulder to pin) (cm.)

(f) Hip-hip (cm.)

(g) Hip-pin (cm.)

In all cases an average of five weekly measurements, centred about the age recorded, have been used in the analysis. Milk weights were recorded daily and an a.m. p.m. composite milk sample tested daily for fat content, except that a combined representative sample was taken for Saturdays and Sundays.

RESULTS

Growth

Of the 15 original sets in each group, one fraternal and one identical twin member died at an early age. Thus, the growth comparisons are based on 14 sets of identical and 14 sets of fraternal twins. Variance of the growth data have been analysed at 12-weekly intervals during the first two years, components of variance being tabulated in Tables 1-7.

TABLE 1. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

LIVEWEIGHT

Age (weeks)	Type of Twin	Mean (lb.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	169.1	47.0	550.8	597.8	550.8	17.2
	F	148.8	55.6	330.5	386.1	661.0	
24	I	282.3	174.1	1313.0	1487.1	1313.0	113.4
	F	267.5	230.8	622.3	853.1	1244.7	
36	I	351.2	349.4	3964.9	4314.3	3964.9	479.0
	F	334.4	588.9	724.5	1313.4	1449.0	
48	I	396.0	715.4	3020.3	3735.8	3020.3	—
	F	387.4	683.2	1225.1	1908.4	2450.2	
60	I	434.1	660.9	3481.9	4142.8	3481.9	645.2
	F	490.9	983.5	3061.1	4044.6	6122.2	
72	I	579.1	889.8	4362.3	5252.2	4362.3	—
	F	569.4	867.6	3683.4	4550.9	7366.8	
84	I	659.5	662.6	4640.7	5303.3	4640.7	782.0
	F	661.0	1053.6	4732.1	5785.7	9464.2	
96	I	638.2	1130.8	5679.3	6810.1	5679.3	512.6
	F	633.1	1387.1	4967.3	6354.4	9934.6	

NOTE: In all tables the following nomenclature applies:

I Identical twins

F Fraternal twins

V_W Within-set variance component

V_B Between-set variance component

V_T Total variance

V_G Genetic variance

V_{GFI} Genetic variance derived as $2(V_{WF} - V_{WI})$

Growth data after the two-year stage is not reported, for several sets from each group had to be dropped from the experiment when the animals calved, owing to a widespread infection of *Leptospira pomona*, whilst the subsequent conception dates of the remaining animals were far from uniform.

The growth pattern in all animals was very similar although from Table 1 it may be noted that some sets withstood the nutritional check which occurred during the winter months, when the animals were approximately 36-48 weeks of age, very much better than did other sets. In only two sets, T.297 and 298, and F.3 and 4, did the bodyweight difference of the two animals in each set exceed 100 lb. at 96 weeks of age. It is of interest to note that both these particular sets were subsequently very uniform in milk and fat production.

Production

Ten sets of identical twins and eleven sets of fraternal twins successfully completed one lactation. A summary of the lactation yield of each animal is recorded in Tables 8 and 9. The dissimilar milk yield

TABLE 2. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

CHEST GIRTH

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	97.0	2.01	22.0	24.0	22.0	.8
	F	93.9	2.41	14.7	17.1	29.4	
24	I	114.7	2.88	24.1	27.0	24.1	1.8
	F	113.3	3.78	11.4	15.2	22.8	
36	I	124.6	4.49	45.7	50.2	45.7	4.6
	F	124.3	6.82	10.0	16.8	20.0	
48	I	131.2	8.40	50.1	58.5	50.1	3.5
	F	131.1	6.66	12.8	19.4	25.6	
60	I	139.5	7.97	27.3	35.3	27.3	2.0
	F	140.3	6.97	31.8	38.8	63.6	
72	I	150.4	7.00	34.2	41.2	34.2	.6
	F	150.2	7.31	18.7	26.0	37.4	
84	I	157.8	6.84	30.1	36.9	30.1	7.5
	F	158.7	10.61	26.0	36.6	52.0	
96	I	163.1	9.08	45.4	54.5	45.4	1.1
	F	163.2	9.65	20.0	29.6	40.0	

TABLE 3. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

BELLY GIRTH

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	109.7	2.2	26.5	28.7	26.5	6.2
	F	106.6	5.3	20.8	26.2	41.6	
24	I	139.2	6.7	36.6	43.4	36.6	6.0
	F	140.0	9.7	22.5	32.2	45.0	
36	I	151.1	9.9	75.9	85.8	75.9	8.0
	F	152.7	13.9	21.5	35.4	43.0	
48	I	155.9	12.5	49.7	62.2	49.7	9.6
	F	156.8	17.3	14.9	32.1	29.8	
60	I	167.0	11.7	32.7	44.4	32.7	3.4
	F	168.6	13.4	54.5	67.9	109.0	
72	I	178.5	9.5	35.7	45.3	35.7	14.4
	F	180.0	16.7	37.8	54.5	75.6	
84	I	188.0	11.2	42.5	53.7	42.5	10.8
	F	192.2	16.6	51.4	67.9	102.8	
96	I	197.4	9.8	58.1	67.9	58.1	16.4
	F	201.3	18.0	32.6	50.6	65.2	

TABLE 4. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

BODY HEIGHT

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	82.1	1.05	4.68	5.93	4.68	1.42
	F	79.6	1.96	4.13	6.09	8.26	
24	I	94.3	1.46	5.32	6.78	5.32	1.08
	F	92.6	2.00	1.79	3.79	3.58	
36	I	100.2	1.60	6.74	8.34	6.74	2.60
	F	98.8	2.90	3.02	5.92	6.04	
48	I	104.0	2.10	6.73	8.83	6.73	1.56
	F	103.7	2.88	2.36	5.24	4.72	
60	I	107.9	1.45	8.36	9.81	8.36	2.18
	F	107.7	2.54	2.03	4.57	4.06	
72	I	111.7	1.15	9.41	10.56	9.41	3.14
	F	111.1	2.72	2.10	4.82	4.20	
84	I	114.9	1.20	8.66	9.86	8.66	2.44
	F	114.9	2.42	3.45	5.87	6.90	
96	I	117.6	1.65	7.65	9.30	7.65	.66
	F	117.4	1.98	5.23	7.21	10.46	

TABLE 5. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

BODY LENGTH

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	88.9	1.07	7.0	8.1	7.0	11.92
	F	85.6	7.03	10.3	17.3	20.6	
24	I	108.1	2.13	12.2	14.3	12.2	9.98
	F	105.4	7.12	7.7	14.8	15.4	
36	I	116.9	2.41	17.0	19.4	17.0	16.2
	F	114.7	10.42	8.8	19.2	17.6	
48	I	121.6	12.48	32.0	44.4	32.0	1.48
	F	120.0	11.74	6.8	18.5	13.6	
60	I	129.7	2.87	14.7	17.6	14.7	8.38
	F	127.4	7.06	16.2	23.3	38.4	
72	I	136.5	5.32	12.8	18.1	12.8	6.26
	F	135.5	8.45	10.5	18.9	21.0	
84	I	141.1	3.47	11.0	14.4	11.0	9.18
	F	140.6	8.06	13.4	21.4	26.8	
96	I	145.4	4.99	15.0	20.0	15.0	7.06
	F	134.2	8.52	13.9	22.4	27.8	

TABLE 6. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

HIP—HIP

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	22.4	.22	1.14	1.36	1.14	.22
	F	21.3	.33	1.24	1.57	2.48	
24	I	28.4	.42	1.53	1.95	1.53	.08
	F	27.6	.38	1.22	1.60	2.44	
36	I	32.0	1.86	2.66	4.52	2.66	2.14
	F	31.3	.79	1.28	2.07	2.56	
48	I	34.2	1.12	2.80	3.92	2.80	.32
	F	33.9	.94	1.96	2.90	3.92	
60	I	37.5	2.89	4.08	6.97	4.08	3.90
	F	37.1	.94	2.47	3.41	4.94	
72	I	39.9	.91	3.53	4.44	3.53	.10
	F	39.6	.96	2.11	3.07	4.22	
84	I	42.1	.74	3.56	4.30	3.56	.02
	F	42.1	.75	2.63	3.38	5.26	
96	I	44.3	.82	3.94	4.76	3.94	.72
	F	44.2	1.18	2.45	3.63	4.90	

TABLE 7. IDENTICAL- FRATERNAL-TWIN GROWTH DATA

HIP—PIN

Age (weeks)	Type of Twin	Mean (cm.)	V_W	V_B	V_T	V_G	V_{GFI}
12	I	28.2	.13	1.25	1.38	1.25	.48
	F	27.1	.37	.75	1.12	1.50	
24	I	33.2	.22	1.73	1.95	1.73	.54
	F	32.6	.49	.33	.82	.66	
36	I	36.0	.31	3.10	3.41	3.10	1.14
	F	35.5	.88	.34	1.22	.68	
48	I	37.8	.84	2.60	3.44	2.60	.16
	F	37.6	.76	.66	1.42	1.32	
60	I	40.3	.76	1.92	2.68	1.92	.16
	F	40.2	.68	1.26	1.94	2.52	
72	I	42.4	.65	2.32	2.97	2.32	.04
	F	42.1	.67	.98	1.65	1.96	
84	I	44.1	.50	2.26	2.76	2.26	.38
	F	43.9	.69	1.65	2.34	3.30	
96	I	45.4	.63	2.63	3.26	2.63	.32
	F	45.2	.79	1.60	2.39	3.20	

TABLE 8. IDENTICAL-TWIN PRODUCTION DATA

	Fat	Milk	Test	Days	Calving Date
T297	230	5618	4.10	258	26.9.53
T298	229	5399	4.24	304	12.7.53
T299	196	4132	4.74	265	30.7.53
T300	186	3968	4.69	256	20.7.53
T309	178	3988	4.60	246	30.7.53
T310	111	2534	4.39	175	29.7.53
T311	299	6504	4.59	301	6.8.53
T312	357	7823	4.56	305	20.7.53
T313	21	407	5.11	117	31.7.53
T314	16	336	4.81	77	9.9.53
T317	208	5980	3.48	295	19.8.53
T318	222	6330	3.52	250	4.10.53
T321	202	4356	4.64	305	10.8.53
T322	219	4887	4.49	305	8.7.53
T327	309	6709	4.61	305	19.7.53
T328	307	6801	4.51	304	12.7.53
T329	256	5002	5.11	298	17.8.53
T330	267	5244	5.10	305	4.8.53
T331	159	3723	4.27	230	15.8.53
T332	242	5459	4.42	298	17.8.53

TABLE 9. FRATERNAL-TWIN PRODUCTION DATA

	Fat	Milk	Test	Days	Calving Date
F 1	24	496	4.92	138	17.7.53
F 2	273	4966	5.51	304	29.7.53
F 3	281	6150	4.56	305	27.7.53
F 4	296	6787	4.36	302	22.7.53
F 9	284	5468	5.19	302	28.7.53
F10	283	5468	5.18	283	19.7.53
F13	325	5997	5.42	305	24.7.53
F14	294	5567	5.29	305	18.7.53
F15	116	2478	4.69	167	11.8.53
F16	102	2001	5.11	189	20.7.53
F17	222	5271	4.22	303	27.7.53
F18	38	856	4.38	139	16.7.53
F19	314	8678	3.62	305	7.7.53
F20	298	8908	3.34	305	14.7.53
F21	59	1144	5.17	162	28.7.53
F22	54	1015	5.34	157	2.8.53
F23	172	3713	4.64	258	13.8.53
F24	205	4177	4.90	265	6.8.53
F25	209	3501	5.97	234	2.8.53
F26	288	5245	5.48	278	24.7.53
F29	215	4222	5.10	268	3.8.53
F30	168	3614	4.65	270	6.8.53

of Twins T.309 and T.310 was primarily due to an udder infection in T.310, whilst in the case of T.331 and T.332 it is considered that infection with *Leptospira pomona* may have been a contributory factor to the uneven yields. In the case of the fraternal twins, no explanation, apart from varying genotype, can be provided to explain the divergent yields. All animals have been included in the analyses.

Table 10 lists the components of variance for fat and milk yield and fat test.

TABLE 10.

Butterfat Production

Type of Twin	Mean	V_W	V_B	V_T	V_G	V_{GFI}
I	210.7	768	7166	7934	7166	8226
F	205.5	4881	5214	10095	10428	

Milk Production

Type of Twin	Mean	V_W	V_B	V_T	V_G	V_{GFI}
I	4760	370,948	3,642,604	4,013,552	3,642,604	3,256,956
F	4351	1,999,426	3,730,950	5,730,376	7,461,900	

Butterfat Test

Type of Twin	Mean	V_W	V_B	V_T	V_G	V_{GFI}
I	4.50	.01069	.19135	.20204	.19135	.08994
F	4.86	.05566	.34902	.40468	.69804	

DISCUSSION

With identical twins, two sources of variation only may be distinguished in uniformity trials that are not time replicated: between-set differences and within-set differences. The between-set variance contains not only all genetic effects—both additive and non-additive—but also includes any genotype-environment interaction present. Thus, the ratio genetic variance/total variance (V_{GI}/V_T) is likely to be high.

In the case of fraternal twins, estimates of genetic varience (V_{GF}) based on the assumption that twin members will have 50% of genes in common, are again likely to overestimate the true additive genetic variance. This situation comes about as a result of genetic and genotype-environment interactions due to genes held in common by the twin pairs.

With both the identical and fraternal twins, the within-set variation tends to be reduced by non-inherited effects such as a similar

pre-natal environment, contemporaneity, and similar local environment. As Lush pointed out[5] the within-set variation of the fraternal twins contains half the total additive genetic variation, together with most of the non-additive genetic variation, whereas the within-set variation in identical sets contains error variation only. Thus, a comparison of the within-set variation of the fraternal sets with the within-set variation of the identical sets would lead to an estimate of genetic variance (V_{GFI}) uncomplicated by similarities of environment common to the twin sets only but containing most of the non-additive genetic variations. Estimates of the genetic variance derived in this fashion are listed beside the estimates derived for the identical and fraternal animals independently (Tables 1-7).

An inspection of Table 10 reveals that the three possible estimates of genetic variance for milk production and fat production are of approximately similar magnitude, whereas in the growth data (Tables 1-7) the estimate of genetic variance (V_{GFI}) is very much less than either of the other two ($V_{GF} + V_{GI}$). In effect, it appears that pre-natal environment and the contemporaneity of the twins had a marked effect on growth, whereas there was little effect of these two factors on milk production. Since it is unlikely that a contemporary environment is of greater importance to growing than lactating twins, it would seem reasonable to conclude that pre-natal environment is the factor exerting the greatest effect on growth of such animals under uniform conditions and that true additive gene effects exert but a comparatively small influence. It might also be argued that non-additive genetic interactions may be of greater importance to milk production than growth, though the close similarity of heritability and repeatability estimates for milk production suggest only a limited scope for such interactions. Whatever the case, it appears that fraternal twin animals would be a most satisfactory substitute for monozygotic animals in growth studies.

While the suggested explanation appears plausible, it must be accepted with reservation. In the first place, sampling errors of the estimated variance components are very large. Secondly, the estimated total variance of the identical twin data is, in the main, markedly different from that in the fraternal twin data, thus it is hardly valid to compare the respective variance components.

A further point concerns the incidence of foetal anastomoses in both fraternal and identical twins which are believed to occur in some 90% of all twin calvings. From this it might be argued that the marked pre-natal effects already noted are largely due to the common blood supply of most twins prior to birth and not to variations in what may be considered the true intra-uterine environment. Although the estimates of the influence of pre-natal environment reported in this paper are in agreement with recent observations concerning the role of pre-natal influences upon the pre-weaning growth of mice[6], further data are required before this observation may be accepted as substantiated.

The close similarity of the productive records of the identical twins is in accord with earlier findings[1] and implies that, under controlled environmental conditions, the phenotype of an animal is a good index of its genotype and that under such conditions animals of superior breeding worth may be efficiently selected on the basis of phenotype

records—always provided that non-additive gene interactions are not of major importance in determining productive merit. An estimate of the magnitude of such gene interactions has been made but, owing to limitations in the data, already discussed, further information is required to decide the matter.

Finally, it should be noted that the authors are all-too-aware of the deficiencies in the quantity of data available and thus of the soundness of the variance component estimates derived. However, in view of the extremely limited information available in this field, together with the high cost and appreciable time lag in obtaining such data, publication of these results is considered justified as being of general value to other workers particularly since similar small-scale studies are known to be in progress elsewhere.

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RUMEN STUDIES IN SHEEP

I. VARIATION IN RUMEN MICROBIAL END-PRODUCTS IN FREE-GRAZING SHEEP

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Summary

Determinations of the major end-products of microbial digestion have been made on the rumen contents of free-grazing sheep during the spring and summer. Mean levels found were: pH, 6.6; total volatile fatty acids, 10.4 millimoles per cent; ammonium nitrogen, 23.2 mg per cent; protein nitrogen 226 mg per cent; and free microbial count, 69×10^6 per cu. mm.

Variation in these constituents due to differences between sheep, between days, between times during the day, and between two groups on different pastures, has been studied. Although sampling errors were large, observations on individual sheep showed satisfactory repeatability. Levels of constituents were relatively constant during the day, following the morning grazing.

The most practical measure of the mean levels of rumen constituents in a large group of sheep may be obtained by taking single samples from the largest possible number of animals.

Where the number of animals in a group is small, sampling once a day on several days is preferable to sampling more than once daily on fewer days.

Introduction

The end-products of rumen microbial fermentation are known to play an essential part in the nutrition of the host and any radical imbalance among them is liable to result in lowered rate of feed digestion and in physiological disorder. Despite the continuous removal of these end-products by absorption through the epithelium and by passage to the omasum, it seems likely that the concentrations present in the ruminal fluid will reflect their rate of production and may therefore be expected to provide a measure of ruminal microbial activity. The term activity, in this case, represents the interaction of the rumen micro-organisms with the ingested material and does not distinguish between the suitability or quantity of the feed on the one hand, and the adaptation of the flora to the particular diet on the other; it does not indicate the potential capability of the flora to deal with any more nutritious diet that may be provided.

Determinations likely to reveal abnormalities in activity by survey procedures are pH, total volatile fatty acids (VFA), ammonium nitrogen, protein nitrogen (PN), and free microbial count. Gross changes in pH indicate abnormal rumen conditions[1, 2]. VFA are the major end-products of carbohydrate breakdown[3] and provide the main energy source to the host[4]. High levels of ammonia may indicate wasteful use of feed protein, particularly if unaccompanied by rapid carbohydrate fermentation[5, 6, 7]. The PN of the ruminal fluid roughly represents microbial protein nitrogen[6]. This is known to be of high biological value[8, 9]. Even though the conversion of feed to microbial protein in

the rumen cannot be directly measured, the concentration of PN in the ruminal fluid may give some indication of any large differences in this facet of microbial activity. The free rumen microbial population, representing approximately 85% of the total population[10], should indicate the nutrient status of the ruminal fluid for supporting microbial growth[11] and any marked changes in the species of organisms present can be detected by examination of a Gram-stained smear[1, 11]. Vitamin synthesis, which is an important feature of microbial activity[12], cannot be studied on a survey basis.

Little information is available on the levels of these constituents in free-grazing sheep or the variability to be expected. It is also necessary to find the times of maximum activity during the day, since measurements made then may be expected to show the greatest differences in rumen end-products. To investigate the applicability of these techniques to comparisons between animals and between groups of animals, uniformity trials have been carried out as a preliminary to further work.

EXPERIMENTAL

Sampling

Four trials were conducted to study the following sources of variation:

(a) Between sheep

(b) Between days

(c) Between times during the day

(d) Between groups on different pastures

In Trials 1–3, six two-tooth Romney ewes were grazed together in a one-acre paddock of perennial rye/white clover pasture from 14 September to 10 December 1953. In Trial 4, six Romney wether hoggets (Group D) were grazed on dairy pasture from 9 February to 17 February 1954 and six of similar live-weight (Group S) on sheep pasture for the same period. Both dairy and sheep pastures were good-quality mixed swards consisting mainly of perennial rye, white clover, and Yorkshire fog, but paspalum was prominent on the sheep farm only.

Samples of rumen contents (minimum 50 ml) were taken by stomach pump as follows:

Trial 1: at 0700, 1200, and 1700 hours on six days at weekly intervals.

Trial 2: at 1030 and 1300 hours on three days at intervals of several days.

Trial 3: at 1200 hours on four consecutive days per week for two weeks.

Trial 4: at 1030 and 1300 hours on five days over a ten-day period.

All analyses were carried out on the fluid expressed through a single layer of muslin. (Earlier work showed that such samples taken at the same time from the same sheep gave results agreeing to within $\pm$ 5%).

TABLE 1. MEAN LEVELS (ALL TRIALS)

Constituent	Mean and Standard Deviation	Range
Ammonium nitrogen (mg%)	23.2 ± 7.1	9.1 — 46.6
Volatile fatty acids (millimoles %)	10.4 ± 1.5	7.5 — 14.2
Protein nitrogen (mg%)	226 ± 56	103 — 397
pH	6.6 ± 0.3	5.9 — 7.2
Microbial count (x 10^6/cu.mm)	69 ± 18	37 — 143

TABLE 2. MEANS OF LEVELS DURING THE DAY

Constituent	Trial No.	Hour 0700	1030	1200	1300	1700
Ammonium nitrogen (mg %)	1	21.4 ± 7.1	—	27.8 ± 7.5	—	30.9 ± 7.0
	2	—	24.9 ± 8.4	—	21.6 ± 6.0	—
	3	—	—	24.4 ± 5.2	—	—
	4 D	—	19.9 ± 4.2	—	18.8 ± 4.8	—
	4 S		19.6 ± 6.6		20.2 ± 5.6	
Volatile fatty acids (millimoles %)	1	9.4 ± 1.0	—	10.3 ± 2.0	—	10.6 ± 1.7
	2	—	11.6 ± 1.7	—	9.8 ± 0.9	—
	3	—	—	9.6 ± 1.0	—	—
	4 D	—	11.2 ± 1.6	—	10.7 ± 1.4	—
	4 S		10.8 ± 1.8		10.5 ± 1.6	
Protein nitrogen (mg %)	1	281 ± 49	—	255 ± 62	—	224 ± 37
	2	—	233 ± 38	—	194 ± 25	—
	3	—	—	245 ± 47	—	—
	4 D	—	213 ± 64	—	198 ± 53	—
	4 S		193 ± 42		192 ± 48	
pH	1	6.7 ± 0.2	—	6.7 ± 0.4	—	6.6 ± 0.4
	2	—	6.5 ± 0.2	—	6.5 ± 0.2	—
	3	—	—	6.8 ± 0.2	—	—
	4 D	—	6.5 ± 0.2	—	6.5 ± 0.2	—
	4 S		6.6 ± 0.3		6.4 ± 0.2	
Microbial count (x 10^6/cu.mm)		—	—	69 ± 18	—	—

D. = Dairy-farm group S. = Sheep-farm group

TABLE 3. ANALYSIS OF VARIANCE—SEPARATE TRIALS

Trial	Source of Variation	d.f.	Ammonium Nitrogen		Volatile Fatty Acids (VFA)		Protein Nitrogen (PN)		pH (x10)		Free Microbial Count (x10⁶cu.mm)	
			m.s.	v.c.	m.s.	v.c.	m.s.	v.c.	m.s.	v.c.	m.s.	v.c.
1	Sheep (S)	5	81.6**	3.4	4.02*	0.16	5,682**	235	22.6	—		
	Days (D)	5	484.2	17.4	22.86	0.87	14,113	512	96.6	3.86		
	Times (T)	2	844.1*	19.0	13.70	0.20	28,394*	652	18.0	—		
	D × T	10	160.0**	25.3	6.56**	1.00	4,892**	574	24.5	3.62		
	S × D	25	19.8**	3.9	1.15**	0.21	1,395		5.4	0.87		
	S × T	10	8.6	8.1	0.39	0.53	1,259	1,448	3.2	0.07		
	S × D × T	50	8.0		0.56		1,513		2.8	2.80		
2	Sheep (S)	5	100.0**	15.0	4.48**	0.60	1,884	128	22.8	0.60		
	Days (D)	2	532.0	40.9	6.80	0.35	537	—	13.0	0.08		
	Times (T)	1	96.0	3.0	30.40	1.54	7,715	274	0.0	—		
	D × T	2	39.2*	5.6	2.60	0.29	2,553*	324	0.6			
	S × D	10	7.8	1.2	0.97		894	143	1.3	1.04		
	S × T	5	7.8	0.8	0.30	0.87	833	75	0.2			
	S × D × T	10	5.5	5.5	1.06		609	609	1.1			
3	Sheep (S)	5	54.4	4.1	2.79	0.17	7,053	584	9.8	0.58	2499.1	—
	Weeks (W)	1	24.5	—	0.01	—	4,800	—	42.0	—	392.8	—
	Days (within Weeks) (D)	6	94.2**	14.2	1.19	0.10	4,126**	541	5.2	0.55	663.8	—
	S × W	5	22.0	3.2	1.42	0.20	2,380*	376	11.2	1.37	9438.5	6574.3
	S × D	30	9.2	9.2	0.61	0.61	878	878	3.0	3.00	2864.2	2864.2
4	Groups (G)	1	9.9	—	0.49	—	4,980	—	0.0	—		
	Days (D)	4	59.8	—	6.77	—	4,330	101	22.5	0.25		
	Times (T)	1	2.1	—	1.47	—	2,042	10	1.0	—		
	Sheep (within Groups) (S)	10	112.8**	10.0	10.74**	0.81	21,365**	1,997	18.7	1.37		
	D × T	4	56.7	2.8	4.00**	0.28	308	—	15.8	0.22		
	G × D	4	196.2*	14.4	5.67*	0.32	637	—	14.0	—		
	G × T	1	19.4	—	1.96	0.02	1,462	15	4.0	—		
	G × D × T	4	23.2	2.3	0.45	(1)	1,013	86	13.2	1.83		
	S × D	40	9.5	(1)	1.88**	0.60	1,392**	448	2.6	0.35		
	S × T	10	13.2	0.7	1.40*	0.14	429		4.3	0.48		
	S × D × T ("Error")	40	9.4	9.5	0.71	0.69	512	496	1.9	1.90		

d.f. = degress of freedom; m.s. = mean square; v.c. = variance component.
(1) included in "Error"

* significant ($P < 0.05$)
** highly significant ($P < 0.01$)

TABLE 4. POOLED (APPROXIMATE) ANALYSIS OF VARIANCE—ALL TRIALS

Source of Variation	d.f.	Expectation of mean squares	Ammonium Nitrogen (mg %)			Volatile Fatty Acids (millimoles %)			Protein Nitrogen (mg %)			pH (x10)		
			m.s.	v.c.	%	m.s.	v.c.	%	m.s.	v.c.	%	m.s.	v.c.	%
Sheep (S)	25	$\sigma_{SDT}^2 + 2\sigma_{SD}^2 + \frac{27}{5}\sigma_{ST}^2 + \frac{52}{5}\sigma_S^2$	92.32	7.49	14.1	6.56	0.50	18.6	11,352	970	33.0	18.52	1.17	4.5
Days (D)	22	$\sigma_{SDT}^2 + 6\sigma_{DT}^2 + \frac{21}{11}\sigma_{SD}^2 + \frac{126}{11}\sigma_D^2$	231.80	11.18	21.0	8.40	0.30	11.2	5,048	159	5.4	38.36	19.06	73.8
Times (T)	5	$\sigma_{SDT}^2 + 6\sigma_{DT}^2 + 5\sigma_{ST}^2 + 30\sigma_T^2$	361.34	8.65	16.2	12.24	0.26	9.7	13,601	355	12.1	10.00	—	-
D × T	20	$\sigma_{SDT}^2 + 6\sigma_{DT}^2$	99.86**	15.26	28.6	4.43**	0.63	23.4	2,966**	330	11.2	18.20	2.66	10.3
S × D	110	$\sigma_{SDT}^2 + \frac{21}{11}\sigma_{SD}^2$	12.18*	2.02	3.8	1.26**	0.31	11.5	1,252	139	4.7	3.35	0.52	2.0
S × T	25	$\sigma_{SDT}^2 + 5\sigma_{ST}^2$	10.27	0.39	0.7	0.77	0.02	0.7	842	986	33.6	3.04	0.16	0.6
S × D × T	100	σ_{SDT}^2	8.33	8.33	15.6	0.67	0.67	24.9	1,022			2.25	2.25	8.7
Repeatability (eliminating D, T, and D × T.)			0.41			0.33			0.46			0.29		

d.f. = degrees of freedom; m.s. = mean square; v.c. = variance component; **P <0.01; *P <0.05

Methods

Determinations of pH were carried out on a Beckman pH meter, using a glass electrode. VFA were estimated by distilling through a Markham still[13], and ammonia by the method of Conway[14]. PN was calculated as difference between total N and non-protein N, using dilute sulphuric acid as protein precipitant[6]. Free microbial counts were made for Trial 3 only, by the method of Moir[15].

RESULTS

The mean levels of ruminal constituents for all trials, together with the ranges and standard deviations, are presented in Table 1. The mean levels at different times during the day are presented in Table 2. Little difference was found among means for the separate trials; in particular, groups D and S in Trial 4 showed remarkably similar values.

The analyses of variance for the separate trials are shown in Table 3.

DISCUSSION

Because of the nature and duration of the disturbance to the normal grazing pattern caused by yarding, holding, and sampling, it was considered neither practicable nor desirable to sample each sheep more than three times a day or six times a week. Five different times of day were therefore chosen for the various trials in order to obtain a picture of the overall diurnal variation (Table 2). Analysis of variance (Table 3) shows that, in Trials 2 and 4, there was no significant difference between the 1030 and the 1300 hour sampling. In Trial 1, significant trends were found to occur during the day in ammonia and PN, though not in VFA. Comparing Trial 1 with Trials 2 and 4, it appears that ammonia levels were low and PN high at the 0700 hour sampling compared with succeeding samples, which remained relatively constant for the rest of the day. It was sometimes apparent from the fluid nature of the rumen contents that the sheep had not grazed prior to the 0700 hour sampling. The rise in ammonia later in the day may therefore be ascribed to a rapid breakdown of readily available nitrogen in the feed, and the fall in PN to a dilution effect. A similar fall after eating has been previously observed in microbial population (M. C. Nottle, pers. comm.). Since large quantities of fibre remain in the rumen 24 hours after feeding, VFA levels would not be expected to show the same fluctuations as ammonia. Sampling before the morning grazing period should therefore be avoided in studies on activity. With this reservation, it seems that time of day is unimportant in any sampling procedure.

Sampling errors are seen to be large, which is not surprising in view of the continually changing environment obtaining under field conditions. However, differences between sheep over periods of time are relatively consistent for all biochemical constituents, and the repeatability of observations on individual sheep is shown to be satisfactory though replication is obviously advisable.

Group comparisons are therefore feasible, provided at least six animals per group are used and a suitable sampling design is chosen. Clearly the number of samples taken each week must be limited by the interference caused to the animal and by the number of analyses which can be handled in the laboratory.

If a large experimental group is available, it appears preferable, because of the importance of variations due to sheep, to take single samples from as many sheep as possible, rather than repeat samplings on the same animal.

In many experimental designs, however, the number of sheep that can be subjected to each treatment is restricted, and under such circumstances attention is necessarily centred on the relative importance of the sampling errors associated with days and with times of day. An examination of the interactions of sheep with days and of sheep with times, as well as a more rigorous treatment of the data (see Appendix), shows that the best method is to sample once on each day for as many days as possible.

Although no appreciable differences were found between the groups involved in the present study, the techniques may have application in detecting differences of biological importance due to widely varying pasture conditions or to rumen dysfunction.

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STATISTICAL APPENDIX

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In Table 3 are summarized the analysis of variance and the estimates of variance components, the latter being based on the supposition of a fully random model in each case. While the sheep may be regarded as a random sample from a population of different

animals, days and times may in effect be regarded as "fixed" factors, whose effects are eliminated in treatment comparisons, but whose interactions with sheep constitute random effects.

Although the trials all differed in their designs, a joint (approximate) analysis was undertaken (Table 4) in order to obtain the most efficient pooled estimates of the variance components. For this purpose, the two groups of Trial 4 were treated as separate trials, which ignores the fact that the days and times represented were common to both groups; however, the absence in this trial of significant overall day or time effects (Table 3) gives reasonable justification for this procedure. The "Weeks" classification in Trial 3 was ignored (the overall differences between weeks were found to be non-significant); and the pooled analysis is on a "within trial" basis so that the day, time, and day by time components include possible interaction with trials or groups.

Repeatability

Assuming days and times main effects and their interaction to be eliminated, the "repeatability" of a single random observation on an individual sheep is obtained as the ratio

$$R = \sigma_S^2 / (\sigma_S^2 + \sigma_{SD}^2 + \sigma_{ST}^2 + \sigma_{SDT}^2)$$

Using the pooled estimates of the variances (Table 4) we find for pH, R = .29; for NH_3, R = .41; for VFA, R = .33; and for PN, R = .46. In all cases repeatability is satisfactory, but some replication of measurements seems desirable; for example, estimated repeatability of the means of measurement taken on three separate days are .54, .66, .59 and .72 respectively.

Sampling plans

Consider a comparison of two treatment groups (G) each of n sheep (S) observed on p days (D) q times each day (T). Suppose the total number of observations 2npq = 2N say, to be fixed. Denoting the mean difference between the two groups by d, we have

$$2N \text{ var } d = (\sigma_{SDT}^2 + n\sigma_{GDT}^2) + p(\sigma_{ST}^2 + n\sigma_{GT}^2) + q(\sigma_{SD}^2 + n\sigma_{GD}^2) + pq\sigma_S^2$$

The maximum precision in the estimated difference is associated with the minimum variance of d. Two cases arise according as we consider n fixed or not.

With n fixed, the minimum of the above quantity occurs when

$$p(\sigma_{ST}^2 + n\sigma_{GT}^2) = q(\sigma_{SD}^2 + n\sigma_{GD}^2)$$

If G x T and G x D interactions are negligible or proportional respectively to S x T and S x D, we have $p/q = \sigma_{SD}^2 / \sigma_{ST}^2$. Caution is necessary in accepting the sample estimates of the relevant variances, subject as they are to wide sampling variation; this is particularly the case in respect of interaction variances involving groups, on which the available information (Trial 4 only) is meagre. However, since (Table 4) σ_{SD}^2 markedly exceeds σ_{ST}^2 for all three characters, and since the amount of replication possible in practice is limited, it would appear preferable to sample only once per day (q = 1) on several days rather than more than once daily on fewer days.

The minimization with n not fixed leads to solution of the simultaneous quadratic equations

$$p\ (\sigma_{ST}^2 + n\sigma_{GT}^2) = q\ (\sigma_{SD}^2 + n\sigma_{GD}^2) \quad \text{(as before)}$$
$$n\ (\sigma_{GDT}^2 + q\sigma_{GD}^2) = p\ (\sigma_{ST}^2 + q\sigma_S^2)$$

with $npq = N$ (constant). For specific values of the variances an iterative arithmetical solution could be obtained. The magnitude of the observed estimate σ_S^2 suggests that n should be taken considerably greater than p which in turn should exceed q. If now, σ_{GT}^2, σ_{GD}^2, and σ_{GDT}^2 are all negligible we have

$$2N \text{ var } d = \sigma_{SDT}^2 + p\sigma_{ST}^2 + q\sigma_{SD}^2 + pq\sigma_S^2$$

Clearly, for variation in n, p, q with $npq = N$ fixed and n, p, q, integral values, this is a minimum when $p=q=1$, $n=N$. That is, in this event the maximum number of animals should be used with only one observation on each.

GROWTH OF PASTURE SPECIES UNDER CONTROLLED ENVIRONMENT

I. GROWTH AT VARIOUS LEVELS OF CONSTANT TEMPERATURE

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Summary

Plants of perennial ryegrass (*Lolium perenne*), short-rotation ryegrass (*L. perenne* x *L. multiflorum*), cocksfoot (*Dactylis glomerata*) paspalum (*Paspalum dilatatum*), browntop (*Agrostis tenuis*), Yorkshire fog (*Holcus lanatus*), white clover (*Trifolium repens*), subterranean clover var. Mount Barker (*Trifolium subterraneum*) and lotus major (*Lotus uliginosus*) were grown at temperatures of 45°F., 55°F., 65°F., 75°F., 85°F., and 95°F. At each temperature the plants received 12 hours of light per day at an intensity of 2,700 ft. candles.

The optimum temperature for growth of the whole shoot was approximately 85°F. for paspalum, 75°F. for white clover and lotus major, and 65–70°F. for the other species.

With an individual tiller, as distinct from the whole shoot, there was, for all species except paspalum, a wide range of temperature over which there was relatively little change in its rate of growth.

Introduction

In order to understand how well species and strains of pasture plants are adapted to the environments in which they occur naturally, or to which they may be introduced, it is important to measure the effects of temperature, and of light intensity, on their rate of growth and their form of growth. These effects largely determine success in various ecological associations and thus productivity and usefulness under various forms of pasture management.

Quantitative information can now be obtained by growing plants under controlled conditions in which the features of the environment can be varied one at a time. The potentialities of this technique for elucidation of problems of crop production and ecology were first clearly demonstrated by Went[15, 16], with studies of the growth of tomatoes.

The controlled-climate technique has since been employed with equal success to assist studies of desert[7] and range ecology[6]. The work of Hiesey[6] with *Poa* spp. and hybrids illustrates how well controlled-climate studies can supplement field work. In transplant experiments initiated by the Carnegie Institute, California, clones were planted in a series of climates and soils ranging from warm coastal to cool montane. The different species and strains showed big differences in their development in the contrasting environments, but this gave little knowledge concerning the genetically controlled physiological characteristics which determined these differences. As a logical step in analysing the relative importance of the various features of the environment in governing the expression of these characteristics, the comparative growth of selected types was studied under a range of temperature conditions in Went's controlled-climate laboratory. It was found that there were large differences between the types in their optimum requirements for

day and for night temperatures. Also there was quantitative inheritance of these requirements by the hybrids. In some cases the findings for types that had been selected from various environments in Europe and America agreed well with deductions made from a consideration of the climate they originated from. In other cases results suggested that types were adapted to a range of environmental conditions extending considerably beyond those in which they were found naturally.

To provide similar information for the pasture species of major economic importance in New Zealand, four controlled-climate cabinets were constructed at Grasslands Division, Palmerston North. These were based on the same design principles as had been used successfully by the author at Manchester, England[8].

It was decided to study first the effects of different levels of constant temperature on the growth of nine species. The temperatures chosen were 45°F. to 95°F. with 10°F. intervals. Constant temperatures were used because earlier unpublished work by the author had indicated that, for the species studied here, rate of growth was approximately the same, for the given mean temperature, whether the temperature was held constant, or whether there was a 10°F. difference between day and night temperatures. The latter approximates the difference between mean temperatures of the day and of the night met by plants in many New Zealand pastures. The effects of larger diurnal variations in temperature are now being studied in detail.

Strains from eight species and one recent hybridization were chosen for examination. These were certified New Zealand perennial ryegrass (*Lolium perenne* L.), certified New Zealand short-rotation ryegrass (*L. perenne* L. x *L. multiflorum* Lam.), certified New Zealand cocksfoot (*Dactylis glomerata* L.), certified New Zealand browntop (*Agrostis tenuis* Sibth.), Yorkshire fog (*Holcus lanatus* L.) (in the text these are collectively described as the "English" grasses), paspalum (*Paspalum dilatatum* Poir.), certified New Zealand white clover (*Trifolium repens* L.), subterranean clover var. Mount Barker (*Trifolium subterraneum* L.), and lotus major (*Lotus uliginosus* Schkuhr).

Methods

The plants received 12 hours of light per day at an intensity of approximately 2,700 foot candles, and with a spectral energy distribution close to that of sunlight. Variation in voltage of the electricity supply, and in age of lamps, prevented either the light intensity or its spectral composition from remaining absolutely constant. Relative humidity was held between 70% and 80% at all levels of temperature.

All determinations were carried out on young plants grown from a sample of seed considered representative of the strain now commercially available in New Zealand. Clones were not used because the objective of this work was to obtain mean values representative for each strain, as a whole, rather than for a particular genotype selected from it. Closer examination of genetic variation within the various strains should be made later. The use of plants recently grown from seeds also ensured that plants tested in the cabinets were always in the vegetative phase of growth, and that an exponential rate of growth was maintained during the experimental period.

Seed from each of these samples was sown in flats and when the young plants had developed 2-3 tillers for the grasses, or 5-6 leaves on the main stem for clovers, they were transplanted to glazed porcelain pots filled with vermiculite. Each pot was three-quart size, and eight plants, arranged in four matched pairs, were put in each. At transplanting, a few very large and very small plants were discarded, and the remainder were paired for size and for habit of growth. From these pairs a random selection was made for the plants which went into the pots. The vermiculite was watered twice daily with an excess of Hoagland's nutrient solution to ensure at all times an adequate supply of nutrient and moisture. After being held for 7 to 10 days in a glasshouse or outside, to allow establishment in the vermiculite, two pots of each species were transferred to a growth cabinet and the plants were allowed a further 7 to 10 days to adapt their rate and habit of growth to the temperature inside the cabinet. This adjustment period was kept short because earlier work had shown that in these species the adaptive changes occur rapidly with plants in their vegetative phase of growth. To reduce further the effects of previous conditions, older leaves which were approaching senescence, or which differed in form from the leaves developing under the experimental conditions, were removed during the conditioning period. This never amounted to more than a light defoliation.

At the end of the conditioning period in the cabinet one plant of each pair was harvested. After the other plant had grown on for a further 8 to 15 days it was harvested. Thus for each species eight plants were taken up at the first harvest and a further group of eight plants at the final harvest. At each harvest green weight and dry weight of the shoot, tiller numbers, and leaf dimensions were measured. Rate of growth of the whole shoot was calculated from the difference in weight between the two groups of plants, but all other results are derived from measurements made on the group of plants which continued growth till the final harvest.

The period between the preliminary and the final harvests was determined partly by the initial size of the plants and partly by the suitability of the experimental conditions for growth. Generally, the faster the growth the shorter the period. The objective was to harvest the plants before they reached a size that could be expected to cause a decline in their rate of growth, either by mutual shading of the leaves or by crowding between tillers at the base of the plants.

The quantity of tissue formed per day on the main-stem tiller of the grasses was determined in the same manner as previously described[10]. For white clover and subterranean clover, the data presented refer to the quantity of leaf tissue, as distinct from stem tissue, formed on a leading stem, but for lotus the data represent the total for both leaf and stem. The stems of white and subterranean clover grow on the soil surface and are normally not removed when the pasture is grazed or mown, but the leaf petiole of lotus always remains short and many of the stems themselves grow up among the surrounding herbage.

In these experiments the roots of the plants were not examined. Considerable information concerning the effect of temperature on the root development of most of these species has already been obtained [10, 11, 12].

As dry weight of the shoot and tiller numbers increased at an exponential rate during the experimental period, results are expressed as percentage increase per day. However, once a tiller or leading stem has passed its initial stages of development, the growth from the single meristematic centre around the apex of the stem continues at an arithmetical and not an exponential rate. Accordingly the growth rate of the main tiller or stem is expressed as milligrammes increase in weight per day.

For each species, and at each temperature, 3 to 5 groups of plants were grown at different times and in different cabinets. The one exception is subterranean clover at 55°F. where only two groups were tested because of considerable loss of plants from virus infection.

Over the complete temperature range there were usually 26 or more points determined for each species. To these 26 points it was decided to fit polynomials of the form

$$Y = a + b\,t + c\,t^2 + d\,t^3 + \ldots$$

where Y represents the character being studied and t the temperature.

Unweighted polynomials were used since there were only isolated cases in which variation could be seen to be related to temperature. These cases occurred only at extreme temperatures where drastic changes in mean were sometimes accompanied by drops in variation, but this was never consistent enough over the species to warrant weighted fitting.

Omitting the data for the extreme temperatures before fitting would have given curves which could not be expected to extrapolate successfully to estimates at those extremes, and which would be therefore unrealistic.

The characters examined in this way were:

Per cent increase in dry weight per day

Per cent increase in tiller number per day

Weight of tissue formed on a tiller per day

Dry matter per cent

Dry weight per square centimetre.

The order of the polynomials to be fitted was determined by fitting polynomials of successive orders, and testing the significance of the increase in explained variation at each stage, the final order taken being that for which no further significant increase occurred. All species were then fitted by polynomials of the same order. For example, in per cent increase of dry weight per day, 6 of the 9 species gave significant improvement as far as the third degree polynomial, so that cubics were fitted throughout.

In the case of weight of tissue formed on a tiller per day, quartics were significantly indicated in all grasses (cubics only being fitted for the clovers as only one pooled point was determined for each temperature in the latter).

In dry weight per square centimetre quadratics only were fitted.

In all other cases cubics were fitted.

From the fitted curves, improved estimates of the means at each temperature can be given, since the associated information at all other

temperature levels is called into the calculation, and the standard errors of these estimates can be given. This statement should be qualified to the extent that the mathematical relationship assumed is arbitrary, but it is felt that polynomials as high as the 4th order can adequately express quite complicated relationships.

From the fitted curves, optimum temperatures can be determined for the various characters, and the values of the character for these optima computed.

For a number of other measurements taken on the plants, the no-curve fitting was carried out. The data presented are the means for each temperature level together with the pooled standard error of that mean for each species.

RESULTS

The effects of temperature on growth are recorded under the following headings:

Rate of growth

Dry-matter content

Morphology and development.

Rate of Growth

The major feature of interest (Fig. 1 and Table 1) is that the optimum temperature for the growth of the shoot lies between 65°F. and 70°F. for six of nine species examined, viz., perennial ryegrass, short-rotation ryegrass, cocksfoot, browntop, Yorkshire fog and subterranean clover. For white clover and lotus major the optimum temperature is higher, about 75°F., and for paspalum it rises to 85°F.

At the highest temperature used (95°F.), the differences between species were even greater than are indicated by the differences in per cent increment in shoot weight per day. With short-rotation ryegrass, browntop, Yorkshire fog, lotus major, and subterranean clover, a considerable number, in some cases all, of the plants in a group died during the experimental period. Plants of white clover, perennial ryegrass, and cocksfoot formed small bluish-green leaves but remained alive. Plants of paspalum grew vigorously but were very "drawn up".

At 45°F. the rate of shoot growth of the "English" grasses had declined to between 8.5% and 11.0% per day, and that of the other species to lower levels. Over a ten-day period this represents a considerable fall in total production. For instance, the daily rate of shoot growth of perennial ryegrass was 14.2% at 65°F., and 9.8% at 45°F. This results in a 2 to 1 difference in total growth of the shoot in a period of ten days.

Features of importance in comparisons between the species are their comparative rates of growth at particular levels of temperature, and whether or not the growth rate of each species remains relatively constant over a wide range of temperatures. Information on these points is presented in Table 1, Fig. 1, and Fig. 4. Fig. 4 sets out the relative differences between species in total growth of the shoot calculated to be present at the end of ten days of growth at the measured rate of increment per day. This period is approximately the average of the intervals between the first and final harvests in the experiments. It also

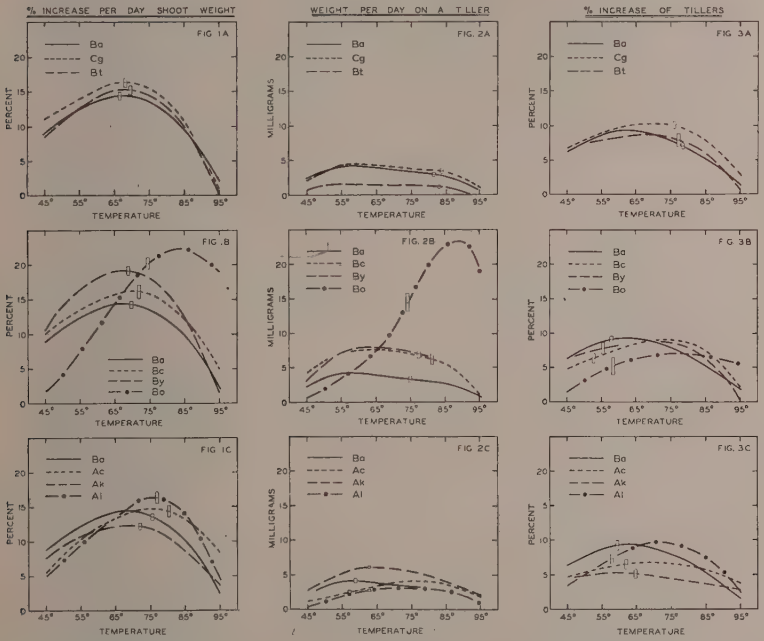
TABLE 1. Estimated Maximum for Rate of Growth of the Shoot, for Growth of an Individual Tiller, and for Rate of Tillering. Estimated Minimum for D.M. Percentage and Dry Weight per Square Centimetre of Leaf Blade. Temperature (°F.) at which these Maxima or Minima are Attained

	Perennial Ryegrass	Short-rotation Ryegrass	Cocksfoot	Paspalum	Browntop	Yorkshire Fog	White Clover	Subterranean Clover	Lotus Major
Maximum percentage increase in dry weight of shoot per day	14.3 68°	16.7 69°	16.1 70°	22.5 85°	15.4 69°	19.2 68°	14.7 75°	12.0 66°	16.4 76°
Maximum weight of tissue formed on a major stem per day (mg)	4.93 57°	3.3 59°	9.1 62°	28.3 88°	1.8 55°	9.3 62°	5.1 78°	7.3 65°	3.7 75°
Maximum percentage increase in tiller number per day	9.3 62°	10.4 71°	9.3 74°	7.1 77°	8.7 69°	8.9 70°	6.7 70°	5.1 58°	9.7 72°
Minimum dry matter percentage of total herbage.	13.2 64°	11.9 68°	13.0 70°	11.0 89°	16.9 63°	11.8 67°	10.9 71°	14.1 71°	12.9 67°
Minimum dry weight per sq. cm. of leaf blade (mg)	2.12 72°	1.60 71°	1.83 69°	1.60 86°	1.46 73°	1.54 68°			

represents an average growth interval under rotational grazing in the field. These estimations allow a clearer expression of the yield differences between species, which result from the exponential rate of increase in shoot weight.

Of the English grasses, the fastest growth was given by Yorkshire fog at 65°F. Its rate of growth remained high at all temperatures from 55°F. to 85°F. Both short-rotation ryegrass and cocksfoot grew faster than perennial ryegrass over a wide range of temperatures. The advantage of short-rotation over perennial ryegrass was greatest at the lower temperatures, and of cocksfoot at the higher temperatures.

With the legumes, the rate of growth of both white clover and lotus major was lower than that of perennial ryegrass at the lower temperatures but above it at the higher temperatures. Although subterranean clover had a low maximum rate of growth, the rate was comparatively uniform over wide range of the lower temperatures.



Ac white clover.
Ak subterranean clover.
Al lotus major.

Ba perennial ryegrass.
Bc cocksfoot.
Bo paspalum.

Bt browntop.
By Yorkshire fog
Cg short-rotation ryegrass.

Fig. 1. Fig. 2. Fig. 3.

Fig. 1.—Percentage daily increase in dry weight of the total shoot.
Fig. 2.—Daily increment (mg.) in dry weight of a leading tiller or stem.
Fig. 3.—Percentage increase per day in tiller numbers.

The rate of growth of the complete shoot is determined partly by the rate of tissue formation on each individual tiller and partly by the rate of increase in number of tillers on a plant. Fig. 2 shows the amount of tissue formed per day on the main tiller or stem, and Fig. 3 shows the percentage increase per day in number of tillers or stems on a plant.

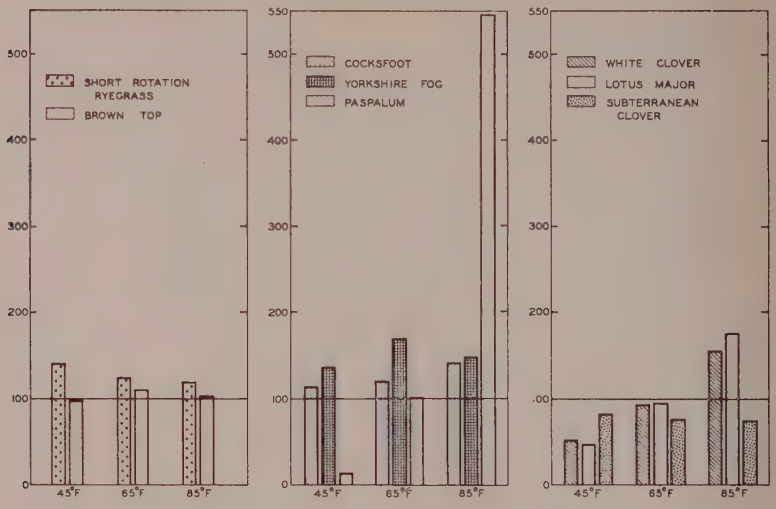


Fig. 4.—Increase in dry weight of shoot over a period of 10 days, relative to perennial ryegrass (100), at temperatures of 45°F., 65°F., and 85°F. The actual percentage increase in weight of the shoot of perennial ryegrass over a period of 10 days was 135 at 45°F., 278 at 65°F., and 195 at 85°F.

Differences between species in the amount of tissue formed per day at optimum temperatures by individual tillers are determined partly by the rate of appearance of successive leaves and partly by the weight of the leaves (Figs. 9 and 10).

With the English grasses the results indicated that the temperature optimum for growth of the individual tiller was lower than for growth of the whole shoot. Also changes in temperature levels, within the range 55°F. to 85°F., had a smaller influence on growth of the individual tiller than on growth of the whole shoot. As the weight of the individual tiller increased at an arithmetical rate and the weight of the whole shoot at an exponential rate, these differences became more noticeable where comparisons are made for a period of ten days.

The high rate of tillering of short-rotation ryegrass at 65°F. and 75°F., and of cocksfoot at 75°F. and 85°F., was largely responsible for these species having faster growth of the whole shoot than did perennial ryegrass at the same temperatures. By contrast, the fast growth of paspalum at the high temperatures arose largely from a spectacular increase in growth of the individual tiller.

Dry-matter Content

Both dry-matter percentage and dry weight per square centimetre of leaf blade (Figs. 5 and 6) were at a minimum at temperatures near the optimum for shoot growth and were higher at both lower and higher temperatures. Hence the rates of increase in dry weight of the whole shoot, at temperatures well above or below the optimum, were greater than were suggested by the appearance of the plants.

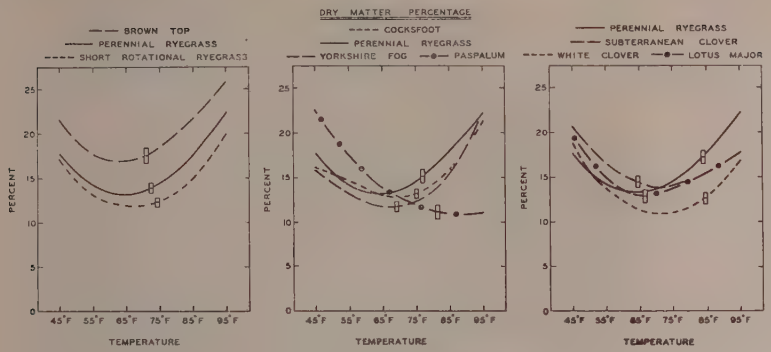


Fig. 5.—Dry matter percentage of whole shoot.

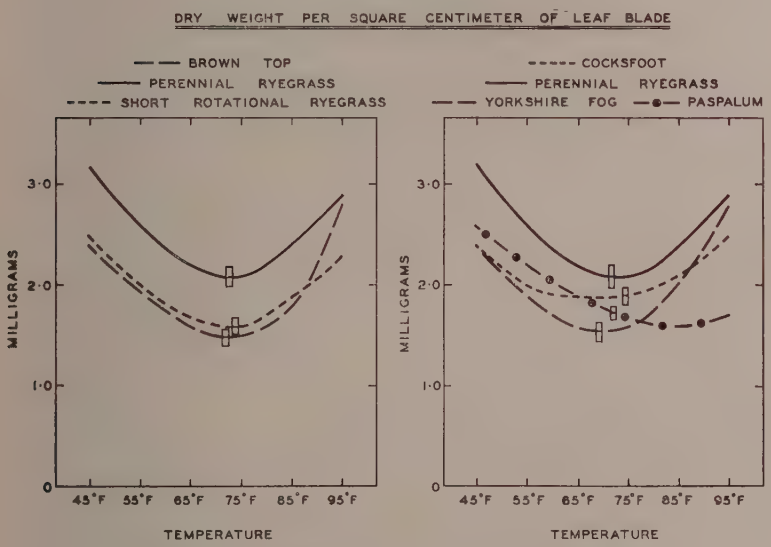


Fig. 6.—Dry weight (mg.) per sq. cm. of the leaf blade.

At all levels of temperature the dry-matter percentages of the herbage of browntop were considerably above those of the other English grasses. For example, at 65°F., browntop contained 28% more dry matter than perennial ryegrass and 42% more than short-rotation ryegrass. The dry-matter percentages of short-rotation ryegrass, Yorkshire fog, and white clover were generally below that of perennial ryegrass. The dry weight per square centimetre of leaf blade was consistently higher in perennial ryegrass than in the other species of grass.

Pattern of Morphological Development

The influence of temperature on the form and weight of the leaves, their rate of appearance on individual tillers, and the total leaf elongation

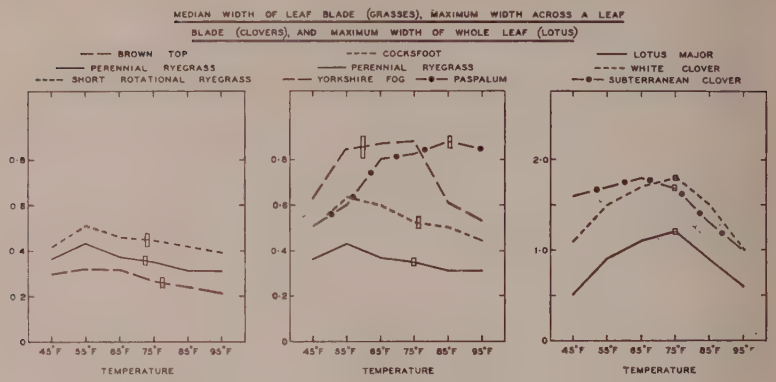


Fig. 7.—Median width of the leaf blade of the grasses. For white clover and subterranean clover the width of the middle leaflet was measured, but for lotus the total width of the leaf was measured.

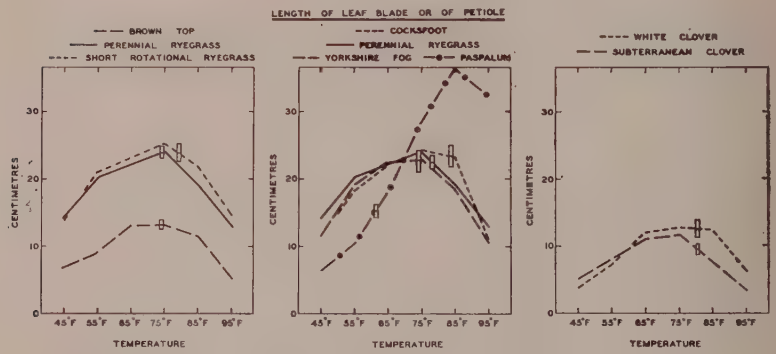


Fig. 8.—Length of leaf blade (grasses) or petiole (white clover and subterranean clover).

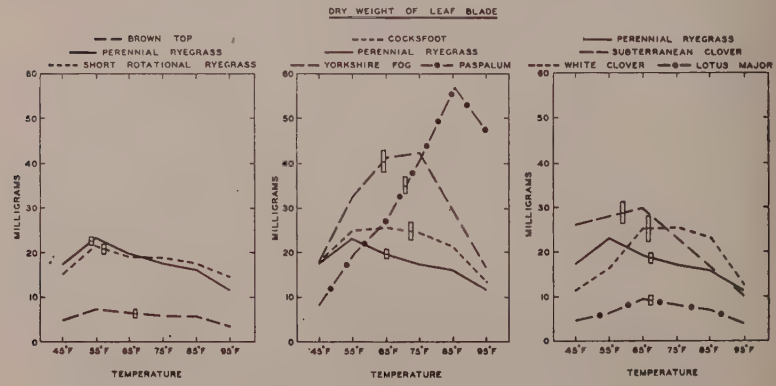


Fig 9.—Dry weight of leaf blades (grasses) or total leaf, i.e., blades plus petiole, and for lotus plus stipules.

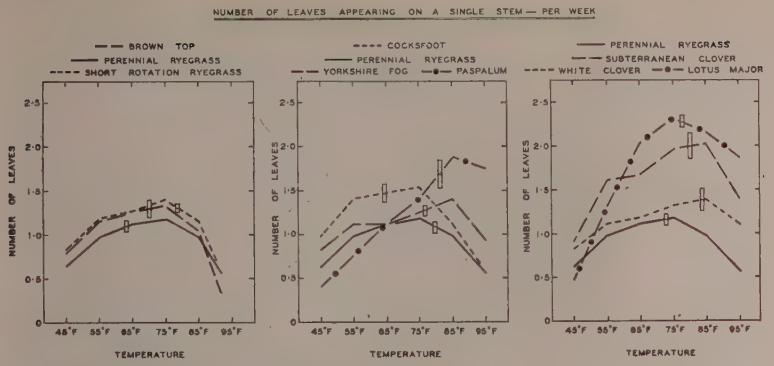


Fig. 10.—Number of leaves appearing on a single stem per week.

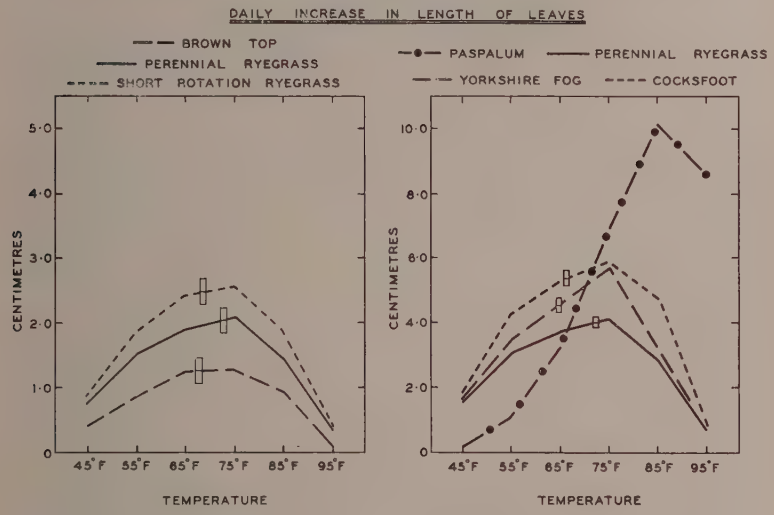


Fig. 11.—Total daily elongation of leaves growing on the main stem tiller.

on a tiller is shown in Figs. 7 to 11. These data are all relevant to ecological studies of competition between the various species, and to studies of the influence of environmental factors on the patterns of morphological development; they will be commented on in a later paper.

Discussion

It is probable that the conclusions drawn from these data may be modified as further information becomes available concerning the influence of other environmental factors on the growth of these species. In particular it is important to measure the effects of different quantities of light, and of large variations in day and night temperatures.

Comparison of these data with the results of previous studies of the influence of temperature on the growth of these species is difficult.

Where attempts were made to control temperature, the light intensity used was generally low[14] or varied with season of the year. However in one case where conditions were reasonably comparable, Brown[3] reported an optimum temperature of 70°F. for the growth of an American strain of cocksfoot. This temperature is the same as that found in these studies for the New Zealand strain.

The present data are complementary to, rather than directly comparable with, statistical evaluations from field data of the influence of the various parameters of the daily weather [1, 2, 5]. Because of the complexity of the daily weather patterns, such statistical evaluations assess trends rather than specific optima or rates of growth at specific levels of temperature and light intensity. Also in such field studies measurements of temperature were generally taken from thermometers set about 4 ft. above the ground in a standard meteorological screen. Temperatures at these levels may be very different from those experienced by a pasture plant growing close to the soil surface[4].

The conclusion from the data reported here—that the optimum temperature for growth of the whole shoot is about 65–70°F. for the English grasses and subterranean clover, about 75°F. for lotus major and white clover, and about 85°F. for paspalum—has to be viewed against the knowledge that in summer the temperature of the surface inch or two of soil in most parts of New Zealand may rise for a considerable period each day to 80°F. or much higher. From an examination of the effects of different levels of soil and air temperatures on the growth of a number of grass species, Brown[3] concluded that the soil temperature near the surface, rather than air temperature, was for those grass plants the major temperature regulator of growth. Similar conclusions have been deduced by Soper and Mitchell[13] who pointed out that in perennial ryegrass the meristematic centres, from which shoot tissue is formed and at which all roots are initiated, are generally placed close to the soil surface, and probably are at a temperature close to that of the adjacent soil. This suggests that the effective temperature conditions in a pasture can often rise to levels where the growth, particularly of the English grasses, is severely checked.

Although it would seem on first thoughts that the rate of growth, as measured by percentage increases in dry weight of the whole shoot of individual plants, is the best index of the productive potential of a species or strain, there is some doubt whether this is always the case. In the present experiments the plants were grown under conditions that encouraged development of tillers and other types of axillary shoots. Rapid increase in tiller numbers of a species will be of importance in assisting rapid establishment after sowing; or re-establishment after a thinning out of the tiller population by summer drought or heat, by winter "pugging", or by smothering from other species. However, with the potential rate of increase in tiller numbers rising to as high as 8% to 10% per day, a limit must quickly be reached in the number of tillers that can be fitted into an area of pasture. Where the total tiller population in an area becomes relatively stable, as probably occurs in many established pastures, the individual rooted tiller becomes the productive unit. In such situations the temperature curves for the individual tiller should be the best index of the effect of temperature on the growth of a species in a pasture. The data indicate that, for all species except paspalum, there is a comparatively wide range of

temperatures over which the rate of growth of the individual tiller shows comparatively little change. With the English grasses a rapid decline in growth did not occur until temperatures were above 85°F. or below 55°F.

It follows that a large and preferably stable population of tillers per unit area of pasture should allow high production during a large portion of the year. It would also ensure that during periods of close grazing there is still a cover over the soil surface, thus giving interception by plant tissue of all the light. The importance of full light interception has previously been referred to by Mitchell[10] and by Brougham[2].

As there are large differences in the size and productivity of the tillers formed by different species, the numbers of tillers required to give equivalent production from an area of ground varies considerably. The data suggest that at 65°F. an approximately equal weight of leaf tissue could come from 100 tillers of perennial ryegrass, 80 tillers of short-rotation ryegrass, 50 tillers of cocksfoot, or 350 tillers of browntop. Obviously, many modifying factors prevent such exact comparisons of the potentialities of species in the field, but despite this, the general thesis should remain valid.

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THE EFFECT OF LEVEL OF FEEDING UPON THE EFFICIENCY OF UTILIZATION OF SEPARATED MILK BY PIGS

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(Received for publication, 13 April 1956)

Summary

1. Two trials conducted to measure the effect of various levels of separated milk feeding upon efficiency of feed conversion are described.

2. In the first experiment, which covered the growth period of 50 to 110 lb., the pigs were group fed and three levels were compared:
   (a) Feed *ad lib.*
   (b) A reference level, called for convenience the 100% level.
   (c) A ration 75% of (b).

3. It was found that pigs fed *ad lib.* scoured badly, persistently fouled their pens, and were less efficient feed converters than those fed on the 100% level. Similarly those fed at the 75% level were less efficient than those fed at the 100% level.

4. In the second experiment all pigs were individually fed. The experimental period was from 40 to 100 lb. The levels of feeding examined were: 100% level, 75%, 65%, 60%, 55% and 50%.

5. There was a highly significant reduction in efficiency between 40 and 80 lb. liveweight as the level of feeding was reduced. The increase in feed requirement for each 20 lb. growth phase per unit percent reduction in ration level was:

40–60 lb.	23.7 oz.**
61–80 lb.	26.4 oz.**
81–100 lb.	5.6 oz. N.S.
40–100 lb.	60.7 oz.*

6. It was concluded that an optimum level of intake for separated milk for pigs between 40 and 80 lb. liveweight was 2.5% to 3% of bodyweight in terms of T.D.N. or, in terms of gallons of milk, between 3.5% and 4% of liveweight.

7. There was a significant and steady reduction in backfat thickness as the level of feeding was lowered.

8. Pigs fed on a ration consisting entirely of separated milk were unable to achieve levels of intake recommended for animals fed on dry feeds.

Introduction

The major pig-feeding problems in New Zealand are those associated with the seasonal changes in the supply of dairy by-products. Farmers endeavour to meet these changes by concentrating farrowings in the early winter and again in the early summer. In this way, winter-farrowed pigs should be heavy enough and possess sufficient capacity to deal with the peak milk supply, while the early-summer farrowings can be marketed before the end of the season.

If the above farrowing plan is put into effect, the food requirements of the pigs on hand at the beginning of the dairy season will exceed

the supply, with the result that either meal supplements must be used or inadequate rations fed.

On the other hand, if sows are farrowed late in the spring, there is no shortage of feed for the weaned pigs; instead there is the problem later in the season of dealing with a rapidly increasing feed supply, with animals possessing relatively small appetites. This naturally leads to an *ad libitum* feeding system.

Since pig feeding in New Zealand is characterised by such wide differences in level of feeding within seasons, it was decided to examine the effect of various levels of feeding upon growth rate and upon the efficiency of feed utilization.

EXPERIMENTAL METHOD

In this study the objective has been to obtain data on the efficiency of feed utilization of separated milk over a wide range of ration levels from *ab lib.* feeding down. Unfortunately, *ad lib.* feeding of pigs on a sole diet of separated milk cannot be achieved under an individual bail-feeding system. For this reason the project has been conducted in two phases:

1. A group-feeding trial in which *ad lib.* feeding was compared with two lower levels of intake.
2. An individual-feeding trial covering six levels of intake.

The two highest ration levels in this latter trial were the same as the lower two in the group-feeding trial. The rationing system used in both phases of the investigation, apart from that of the groups fed *ad lib.* was based on the feed records of individually fed pigs used in previous experiments. This ration, which has for convenience been referred to as the 100% ration, is shown in Table 1 and the lower levels were expressed as a percentage of this.

TABLE 1. DAILY RATION OF SEPARATED MILK (gal.) ACCORDING TO LIVEWEIGHT

Weight Range (lb.)	Daily Ration (gal.)	Weight Range (lb.)	Daily Ration (gal.)
41–45	1.75	71–75	2.75
46–50	2.00	76–80	2.90
51–55	2.25	81–85	3.00
56–60	2.40	86–90	3.10
61–65	2.50	91–95	3.25
66–70	2.60	96–100	3.40

The experiments were conducted as follows:

Trial 1. Group Feeding

In this trial three ration levels were compared:

(a) *Ad lib.*

(b) 100% level.

(c) 75% level.

Duplicate lots of pigs were used, each lot comprising eighteen pigs from three litters. The six pigs from each litter were allotted on a restricted random basis (sex) to the three treatments.

The trial commenced when the groups averaged 50 lb. liveweight and terminated when they averaged 110 lb. All pigs were weighed twice each week. The rations for the 100% and 75% level were adjusted after each weighing. To prevent the milk becoming stale, the *ad lib.* pigs were allowed to empty their troughs once each day and the trough was then refilled and kept full until approximately the same time the next day.

Trial 2. Individual Feeding

A comparison was made of the growth rate and feed conversion efficiency of pigs fed on the following ration levels: 100%, 75%, 65% 60%, 55%, and 50%.

Eight litters, each of six pigs, were used, the six litter mates being allotted at random over the six treatment groups. The pigs were individually fed in bails. The trial commenced at 40 lb. live weight and was terminated at 100 lb. Separated-milk samples were taken daily and the dry matter was determined. Intakes were then adjusted to a standard dry matter of 8%. Weights were recorded twice each week and rations adjusted after each weighing. All pigs were fed three times per day, the quantities being measured in fluid ounces. At the end of the fattening period the pigs were killed and backfat measurements taken.

RESULTS

Trial 1

The relevant data from the first trial are shown in Table 2:

The first of the two groups fed *ad lib.* was placed on treatment as soon as it reached starting weight. The pigs immediately scoured and within 24 hours their pen and bedding areas were completely fouled. Two pigs suffered an anal prolapse and it was feared that two others would die. The ration, therefore, was reduced to the 100% level for ten days during which time scouring ceased, the pen and bedding were kept clean and the general appearance of the group improved. The ration was then raised slowly until full *ad lib.* conditions were attained, and no further trouble was experienced. This group, however, excreted very liquid faeces throughout the experimental period.

The ration of the *ab lib.* treatment in the second lot was raised more slowly and no substantial increase in daily intake above the 100% level was achieved until this group averaged 60 lb.—true *ad. lib.* feeding being reached at approximately 80 lb. live weight. Both the *ad lib.* groups had distended abdomens, were down on their pasterns, and were persistently filthy; the remaining groups were meticulously clean and active, their faeces being firm with no sign of scouring. Owing to the group-feeding technique employed, the growth rate and feed efficiency

TABLE 2. GROWTH RATE, FEED CONSUMPTION AND EFFICIENCY OF FEED CONVERSION OF PIGS FED ON SEPARATED MILK AT THREE LEVELS

(a) *Ad lib.* (b) 100% level (c) 75% level

	Lot 1			Lot 2		
	(a)	(b)	(c)	(a)	(b)	(c)
No. of pigs	6	6	6	6	6	6
Weight at commencement (lb.)	307	308	309	298	293	295
Weight at finish (lb.)	673	663	661	676	660	662
Weight gain (lb.)	366	355	352	378	367	367
Intake (gal.)	1037	954	1045	1119	1012	1106
Gal. per lb. gain	2.83	2.68	2.99	2.96	2.78	3.01
Gain per day per pig (lb.)	1.15	1.05	0.75	1.18	1.02	0.72
Average daily intake per pig (gal.)	3.26	2.84	2.26	3.51	2.81	2.19

data obtained from this trial cannot be subjected to satisfactory analysis. Two facts are, however, worthy of note. The groups fed *ad lib.* grew faster, but not proportionally faster than those fed on the 100% level. For instance, the two *ad lib.* groups consumed 14% and 24% more per day than the groups fed the 100% ration, grew 9.5% and 14% faster, and ate 5.6% and 6.5% more food for each pound of live-weight gain. On the other hand, the 100% groups ate 25.6% and 28% more per day than the 75% ration groups, grew 40% and 41% faster, and ate 11% and 8% less per pound of live-weight gain. It would appear, therefore, that when separated milk is fed *ab lib.* the efficiency with which it is converted into meat is lower than that obtaining when the ration approaches the comfortable capacity of the pig. Further, efficiency of conversion is impaired when the ration is reduced below this level. This latter point is more thoroughly examined in the second trial.

Trial 2

In considering the results, the growth period has been divided into its three consecutive 20 lb. periods: 40–60; 61–80; and 81–100 lb. Table 3 shows the relevant data for these growth stages:

Small differences did exist in the weight gains recorded for the various treatments. Adjustment of intake for these differences by methods of co-variance had negligible effect and the differences have, therefore, been ignored.

Since the total gains are so similar, the effect of feeding levels is reflected in the actual intakes of the experimental animals. For the growth stages 40–60 lb. and 61–80 lb., and for the whole period 40–100 lb., the differences in intake were found to be statistically significant. Except for the 81–100 lb. growth stage, the intake for constant weight-gain increased more or less steadily with decreasing level of feeding.

TABLE 3. MEAN INTAKE, WEIGHT GAINS, DAYS ON EXPERIMENT, AND MEAN DAILY INTAKE ON SIX TREATMENTS OVER THREE WEIGHT RANGES

Ration Level	100%	75%	65%	60%	55%	50%
No. of Pigs	8	8	8	8	8	8
Weight Range 40–60 lb.						
Mean gain per pig	19.3	20.5	20.5	20.8	19.8	20.1
Mean intake per pig (oz.)	5388	6069	6539	6692	6453	6885
Days to gain 20 lb.	17.9	25.5	31.3	34.6	36.2	42.5
Weight Range 61–80 lb.						
Mean gain per pig	20.7	21.3	19.5	20.0	20.25	20.3
Mean intake per pig (oz.)	7056	7273	7568	7571	8025	8633
Days to gain 20 lb.	17.1	23.2	28.0	30.1	35.1	41.8
Weight Range 81–100 lb.						
Mean gain per pig	20.0	19.5	20.6	19.9	19.25	19.5
Mean intake per pig (oz.)	8138	8023	8828	8650	7799	8540
Days to gain 20 lb.	16.7	21.5	28.0	30.3	29.6	35.8
Total Period 40–100 lb.						
Gain per pig	60.0	61.3	60.6	60.7	59.3	60.0
Mean intake per pig (oz.)	20582	21365	22925	22913	22277	24057
Days to gain 60 lb.	52	70	87	95	101	120
Gallons per lb. gain	2.14	2.17	2.36	2.35	2.35	2.50
Mean daily intake (gal.)	2.47	1.90	1.63	1.50	1.37	1.25

To investigate this trend further, the linear component of the treatment sum of squares was isolated and found to be highly significant for all stages except 81–100 lb. There was little evidence of departure from a linear trend. For each unit percent decline in ration level, the extra feed needed to produce a 20 lb. gain in liveweight was estimated to be:

40–60 lb.	28.7 oz.**
61–80 lb.	26.4 oz.**
81–100 lb.	5.6 oz.
40–100 lb.	60.7 oz.*

** Significant at 1 %
* Significant at 5%

In terms of gross efficiency of feed conversion, it may be concluded that below the 100% level there is a progressive reduction in efficiency which can be stated fairly precisely. In terms of energetic efficiency, however, the nature of the tissue constituting the weight gains is of major importance. In this respect, the percentage of fat in the carcass gives some indication of the relative level of energy storage and in turn the backfat thickness of the pigs on the six treatments provides a fairly accurate indication of the differences in total carcass fat.

TABLE 4. MEAN BACKFAT MEASUREMENTS OF PIGS ACCORDING TO FEEDING TREATMENT

Ration level	100%	75%	65%	60%	55%	50%
No. of pigs	8	8	8	8	8	8
Backfat measurement (1/16 in.)						
At shoulder	17.25	17.1	16.25	15.9	14.75	14.25
At loin	10.1	9.75	9.6	9.25	8.25	7.62
Mean carcass percentage	75.0	74.5	74.7	74.8	74.7	74.3

As shown in Table 4, there was a decrease in the backfat thickness of the pigs on the six treatments as the feeding level was reduced. It may be assumed, therefore, that the total fat content and consequently the total energy stored in the carcasses, declined with feeding level in a manner similar to the gross feed efficiency already discussed. The differences shown in the carcass percentages in Table 4 were not significant.

DISCUSSION

The results of the present experiments indicate that feed efficiency was improved when the intake was reduced below that of the groups fed *ad lib.* (4.2% to 4.5% of body weight) to that of the animals fed at the 100% level (3.4% to 4% of body weight). When intake was reduced further, feed efficiency fell.

Data from a number of experiments, notably those of Ellis and Zeller (1934), Gregory and Dickerson (1952), and Lucas and Calder (1955), have shown that a reduction in the level of daily intake to as low as 2% of bodyweight results in an increase in efficiency of feed conversion, as measured by liveweight gains. It has been suggested that this increased feed economy is due to:

1. An improvement in the digestibility of the ration as the intake is reduced.
2. A reduction in the energy losses due to Specific Dynamic Action (S.D.A.) with the result that a higher proportion of the ration is available as net energy.

On the other hand, a reduced daily intake is accompanied by a lengthening of the fattening period and consequently to an increase in the total maintenance cost.

There should be an optimum level of feeding above which savings in total maintenance through reduction in fattening time will not offset the increased losses from reduction in digestibility and increased S.D.A. Below this optimum, increased maintenance costs will exceed any gains in efficiency through increased digestibility and lowered S.D.A.

There is, therefore, a fairly precise limit to the extent to which the daily intake can be lowered and still show a net gain in efficiency. Experimental evidence indicates that this optimum will vary with the

environmental temperature (Lucas and Calder 1955), the breed of pig (Deighton 1929), and the nature of the food (Brody 1945), and it must be related to the current weight of the animal under consideration.

In the present experiments, the maximum daily intake varied (in gallons of milk) from 4.5% of bodyweight for the *ad lib.* groups to 1.72% of bodyweight for the lowest-ration groups. In terms of Total Digestible Nutrients (T.D.N.) the values were 3.4% and 1.3% of bodyweight respectively. It would appear that maximum efficiency is achieved with separated milk between 40 and 80 lb. liveweight when the daily ration (in gallons) is between 3.5% and 4% of the live weight or, in terms of T.D.N., between 2½% and 3% of bodyweight.

This conclusion is not true, however, over the 81–100 lb. growth stage. Over this stage, there was no significant reduction in efficiency as the ration was reduced below the 100% level. There are three possible explanations for this finding:

(a) The experiment was commenced in the spring and progressed through to the summer. The pigs on the higher rations reached finishing weight in an environmental temperature at least 5° lower than that enjoyed by the slower growing animals. This temperature difference could have influenced the feed requirements.

(b) A "growth debt" could have been accumulated by the pigs on the lower ration levels which resulted in a boost in efficiency in the later stages of growth.

(c) The third possibility and the most likely one is that the major part of the greater fat deposition of the pigs on the higher rations took place during the 81–100 lb. growth stage. This would mean that, whereas the efficiency of liveweight gain was similar for all levels over this period, the greater part of the differences in energetic efficiency between ration levels, as shown by the backfat measurements in Table 4, took place during the 81–100 lb. growth phase. The net effect of lowering the ration after 80 lb. would, therefore, be to lengthen the growth period, reduce the fat content of the carcass, and lower the energetic efficiency of the weight gain.

Bearing in mind the qualifications relating to environmental temperature, breed, and the nature of the feeds used, it is of interest to compare the findings reported here with recommended rations from other countries. This comparison is shown in Table 5.

The Rowett data were obtained from an experiment designed to measure the interaction of ration level and environmental temperature and no attempt was made to arrive at an optimum ration level. The lower ration proved to be the more efficient. The point of interest arising from the present results is that pigs are unable to reach the daily intakes recommended by the National Research Council or by Crampton, or the high ration of the Rowett experiment, when fed separated milk *ad lib.* By allowing pigs free access in an attempt to achieve maximum intake, scours became a real management problem, and are probably an indication of reduced efficiency of digestion, leading to lowered feed economy.

The Rowett low-plane ration is slightly below the most efficient Ruakura ration and more closely approaches the 2% bodyweight found

TABLE 5. RATION LEVELS RECOMMENDED IN OTHER COUNTIES COMPARED WITH THE PRESENT EXPERIMENTS
(Values expressed in lb. T.D.N. as % of liveweight)

Liveweight (lb.)	N.R.C.*	Rowett†		Crampton (1952)	Ruakura	
		High	Low		*Ab lib.*	100%
40		4.6	2.7			2.8
50	4.8	4.3	2.5	4.5	3.3	3.0
60		4.1	2.4	4.3	3.0	3.0
70		3.9	2.2	4.2	3.2	2.8
80		3.7	2.1	4.0	3.4	2.7
90		3.7	2.1	4.0	3.4	2.5
100	4.0	3.6	2.1	4.0	3.4	2.5

* National Research Council
† Lucas and Calder (1955)

most efficient by Ellis and Zeller (1934). It is possible that the nature of the feed used, i.e. grains, as compared with separated milk, is responsible for this difference.

The higher rations recommended by the National Research Council (1953) and Crampton (1952) are presumably based on general farm economics rather than upon simple feed efficiency. Where grain is fed as the basic ration, the extra labour cost of hand-feeding pigs over a prolonged fattening period usually exceeds the cost of the extra feed required as a result of self-feeding.

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